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Evaluation of the Mood-Stabilizing Agent Valproic Acid as a Preventative for Toxoplasmosis In Mice and Activity Against Tissue Cysts in Mice

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ABSTRACT: *Toxoplasma gondii* is a common intracellular protozoan infection of humans worldwide. Severe disease can occur in immunocompromised individuals and the in the fetuses of nonimmune pregnant women. Chronic infection is associated with vision and hearing problems, and functional mental alterations, including schizophrenia. The mood-stabilizing agent valproic acid has been shown to inhibit the development of *T. gondii* in vitro at dosages that are normally achieved in the serum and cerebral spinal fluid of human patients and to have positive effects on the behavior of rats chronically infected with *T. gondii*. The present study was done to examine the in vivo activity of valproic acid against acute toxoplasmosis in mice. Two studies were done with valproic acid given in the drinking water at concentrations of 1.5 mg/ml (Experiment 1) or 3.0 mg/ml (Experiment 2). In a third experiment (Experiment 3), valproic acid was injected intraperitoneally (i.p.) at doses of 200 or 300 mg/kg every 12 hr. Valproic acid was not effective in preventing acute toxoplasmosis. All mice treated with valproic acid died or were killed and did not ($P > 0.05$) live significantly longer than the controls. Tachyzoites were demonstrated in the tissues of infected valproic-acid-treated mice. A fourth study was done to determine if valproic acid has activity against *T. gondii* tissue cysts in chronically infected mice. Mice were chronically infected with the ME-49 strain of *T. gondii* for 8 wk and then treated orally with valproic acid at approximately 6.6 mg/ml (800 mg/kg/day) in the drinking water for 10 wk (amount was varied due to increasing mouse weights). No significant differences ($P > 0.05$) were present in tissue cyst numbers in valproic-acid-treated *T. gondii* chronically infected mice and in mice chronically infected with *T. gondii* but not given valproic acid. Our results indicate that valproic acid, although effective in vitro against *T. gondii* tachyzoites, is not effective as a preventative in mice inoculated with *T. gondii* tachyzoites. Additionally, no activity against tissue cysts was observed in chronically *T. gondii*-infected valproic-acid-treated mice.

Toxoplasma gondii is a protozoan parasite that infects humans and most other warm-blooded animals. Humans become infected by ingesting meat containing tissue cysts or by ingesting sporulated oocysts in the environment. Researchers indicate that there are 1,500,000 cases of toxoplasmosis in the United States each year, and about 15% of those infected have clinical toxoplasmosis (Jones, Kruszon-Moran et al., 2001). The importance of maternal infection and congenital toxoplasmosis has long been recognized (Jones, Lopez et al., 2001). The role of chronic *T. gondii* infection on human health was manifested in the AIDS epidemic, with the numerous cases of toxoplasmic encephalitis due to reactivated infection and clinical toxoplasmic encephalitis. The association of chronic *T. gondii* infection and behavioral changes has come to light over recent years and has been strengthened by many studies in humans, mice, and rats (Holliman, 1997; Webster, 2001). Most notably, the association of chronic *T. gondii* infection and schizophrenia has gained attention (Yolken et al., 2001; Ledgerwood et al., 2003; Torrey and Yolken, 2003; Bachmann et al., 2005; Brown et al., 2005; Wang et al., 2006).

Valproic acid is a mood stabilizer used in the treatment of mental illness, including bipolar disorder and schizophrenia (see Bowden, 2007). It has also been shown to inhibit *T. gondii* reproduction in human fibroblast cell cultures (Jones-Brando et al., 2003; Strobl et al., 2007). The IC₅₀ of valproic acid (sodium salt) in a tachyzoite production assay was 266 µg/ml (Strobl et al., 2007), whereas in an ELISA-based assay the IC₅₀ of valproic acid (free acid) was 4.7 µg/ml and the IC₅₀ of valproic acid (sodium salt) was 4.1 µg/ml (Jones-Brando et al., 2003).

These studies indicate that this drug is active against tachyzoites of *T. gondii*.

Rats chronically infected with *T. gondii* lose their innate fear of cat odor (Berday et al., 2000; Vyas et al., 2007). This makes them easier prey for cats and enhances the transmission of the parasite. The effects of valproic acid on the feline avoidance behavior of chronically infected rats was examined by Webster et al. (2006) and it was shown to help treated *T. gondii*-infected rats (40 mg/kg valproic acid/day orally) retain their innate avoidance of cat smell.

The present study was done to examine the anti-*T. gondii* activity of valproic acid in the prevention of acute toxoplasmosis in mice or activity against the tissue cyst stage in chronically infected mice. Female ICR mice were housed in groups of 5 mice per cage (Experiments 1-3) or 3-4 mice per cage (Experiment 4). Mice in Experiments 1-3 were inoculated subcutaneously with 5×10^3 tachyzoites of the RH strain of *T. gondii* on Day 0. Valproic acid was given in the drinking water 1 day prior to subcutaneous inoculation of mice in Experiments 1 and 2. Valproic-acid-containing water in lightproof water bottles was provided ad libitum for the remainder of the study. Fresh valproic-acid-containing water was provided every 2 days. For dosing considerations, we assumed that each mouse would drink 4 ml of water each day. Saccharin was added at 0.2% (w/v) to mask the flavor of the valproic-acid-treated water.

Experiment 1 contained 10 mice treated with 1.5 mg/ml valproic acid (Groups 1 and 2) in the drinking water supplemented with 0.2% (w/v) saccharin and 10 mice not treated with valproic acid (Groups 3 and 4) (Table I). Saccharin was added at 0.2% (w/v) to 1 group of 5 of these mice (Group 3) given water without valproic acid. Experiment 2 contained 5 mice treated with 3 mg/ml valproic acid in the drinking water (Group 5) and 5 mice not treated with valproic acid (Group 6) (Table II). The mean weight of valproic-acid-treated mice in Experiment 1 was 24 g and in Experiment 2 the mean weight was 22 g. Valproic-acid concentrations in the drinking water provided doses of 250 mg/kg/day (Experiment 1) and 545 mg/kg/day (Experiment 2). These dosages translate to 300 mg/kg and 600 mg/kg for a 20-g mouse, respectively.

Experiment 3 was done to evaluate valproic acid administered intraperitoneally (i.p.) at doses of 200 or 300 mg/kg every 12 hr (Table III). The mean weight of valproic-acid-treated mice was 23 g. Valproic acid was dissolved in sterile saline (0.14 M NaCl solution) and i.p. injections were started 2 days prior to RH strain *T. gondii* infection with 5×10^3 tachyzoites s.c. There were 2 groups (Group 7 and 8) of 5 mice each that received the 400-mg/kg/day total dose and 2 groups (Group 9 and 10) of 5 mice each that received the 600-mg/kg/day total dose. A group of 5 mice (Group 11) were infected controls and treated every 12 hr with i.p. sterile saline only.

Experiment 4 (Table IV) was conducted to determine if valproic acid has activity against *T. gondii* tissue cysts in vivo. Eight mice (4 mice in Group 12 and 4 mice in Group 13) were s.c. infected with 1×10^3 tachyzoites of the ME49 strain of *T. gondii* in 0.5 ml HBSS and left untreated for 8 wk. Three mice (Group 14) were s.c. inoculated with 0.5 ml HBSS and treated similarly. After 8 wk, mice in Groups 12 and 14 were provided drinking water containing 0.2% saccharin and approximately 6.6 mg/ml (amount varied because of mouse weights, which changed during the study) valproic acid for 10 wk to deliver a daily dose of 800 mg/kg. Mice in Groups 12-14 were killed 10 wk after valproic-acid treatment, and their brains were removed. The left half of the brain was homogenized in 2 ml HBSS for 2 min with the use of a stomacher machine (Seward Lab Blender Stomacher 80, London, England). The numbers of tissue cysts in a 50-µl sample of the homogenized brain was determined with the use of light microscopy.

TABLE I. Protocol and results of Experiment 1 on 1.5-mg/ml dose of valproic acid given in the drinking water on acute toxoplasmosis in mice.

Group	Mouse number	Dose of valproic acid*	Day post inoculation died/killed
1	1	1.5 mg/ml	Died 9
1	2	1.5 mg/ml	Died 10
1	3	1.5 mg/ml	Died 11
1	4	1.5 mg/ml	Died 11
1	5	1.5 mg/ml	Died 12
2	6	1.5 mg/ml	Died 10
2	7	1.5 mg/ml	Died 11
2	8	1.5 mg/ml	Died 12
2	9	1.5 mg/ml	Died 12
2	10	1.5 mg/ml	Died 13
3	11	None	Died 10
3	12	None	Died 10
3	13	None	Died 11
3	14	None	Died 11
3	15	None	Died 11
4	16	None	Died 10
4	17	None	Died 11
4	18	None	Died 11
4	19	None	Died 11
4	20	None	Died 12

* Provided continuously in water in lightproof drinking bottle to provide an estimated total dose of 250 mg/kg/day.

The right half of the brain was fixed in 10% neutral buffered formalin solution and processed routinely for histological examination following staining with hematoxylin and eosin.

Impression smears were made from the livers or lungs of mice that died or were killed during the study. They were examined unstained for tachyzoites with the use of an Olympus BH60 microscope equipped with interference contrast optics.

Kaplan–Meier survival analysis was performed with the use of PrismGraphpad version 4.0 on mice in Experiments 1–3. The data were analyzed for statistical significance with the use of the chi-square and log-rank tests with a *P* value of 0.05. Tissue cyst counts from mice in Experiment 4 were examined with the use of a 1-way ANOVA and an unpaired *t*-test with a *P* value of 0.05.

Acute toxoplasmosis occurred in all mice given the RH strain (Tables I–III). Neither oral (Experiments 1 and 2) nor i.p. (Experiment 3) treatment with valproic acid was effective. Deaths occurred in treated mice

TABLE II. Protocol and results of Experiment 2 on 3-mg/ml dose of valproic acid given in the drinking water on acute toxoplasmosis in mice.

Group	Mouse number	Dose of valproic acid*	Day postinoculation died/killed
5	21	3.0 mg/ml	Died 11
5	22	3.0 mg/ml	Died 11
5	23	3.0 mg/ml	Died 12
5	24	3.0 mg/ml	Died 12
5	25	3.0 mg/ml	Died 12
6	26	None	Killed 10
6	27	None	Killed 10
6	28	None	Died 10
6	29	None	Killed 11
6	30	None	Killed 11

* Provided continuously in water in lightproof drinking bottle to provide an estimated total dose of 545 mg/kg/day.

TABLE III. Protocol and results of Experiment 3 examining 2 doses of valproic acid given intraperitoneally every 12 hr on acute toxoplasmosis in mice.

Group	Mouse number	Total dose of valproic acid*	Day post inoculation died/killed
7	31	400 mg/kg	Died 9
7	32	400 mg/kg	Killed 9
7	33	400 mg/kg	Died 10
7	34	400 mg/kg	Killed 10
7	35	400 mg/kg	Killed 10
8	36	400 mg/kg	Died 8
8	37	400 mg/kg	Killed 9
8	38	400 mg/kg	Killed 10
8	39	400 mg/kg	Died 10
8	40	400 mg/kg	Killed 10
9	41	600 mg/kg	Died 9
9	42	600 mg/kg	Killed 10
9	43	600 mg/kg	Died 10
9	44	600 mg/kg	Killed 10
9	45	600 mg/kg	Killed 10
10	46	600 mg/kg	Killed 9
10	47	600 mg/kg	Killed 10
10	48	600 mg/kg	Killed 10
10	49	600 mg/kg	Killed 10
10	50	600 mg/kg	Killed 10
11	51	None†	Killed 9
11	52	None	Killed 10
11	53	None	Died 10
11	54	None	Died 10
11	55	None	Killed 11

* Given intraperitoneally as 200 or 300 mg/kg every 12 hr.

† Sterile saline given intraperitoneally every 12 hr.

from 8 to 13 days after inoculation with tachyzoites and in untreated mice 9–12 days after tachyzoite inoculation (Tables I–III). There was no significant positive effect of valproic-acid treatment on mouse survival (*P* > 0.05). Tachyzoites were seen in tissues of all mice given the RH strain of *T. gondii*.

None of the mice inoculated with tachyzoites of the ME49 strain of *T. gondii* died during the study (Groups 12 and 13). None of the mice

TABLE IV. Protocol and results of Experiment 4 on 6.6-mg/ml dose of valproic acid given in the drinking water* for 10 wk on chronic toxoplasmosis in mice.

Group	Mouse number	Dose of valproic acid	Inoculated with <i>Toxoplasma gondii</i>	Number of tissue cysts
12	56	3.0 mg/ml	Yes	0
12	57	3.0 mg/ml	Yes	1
12	58	3.0 mg/ml	Yes	1
12	59	3.0 mg/ml	Yes	3
13	60	None	Yes	0
13	61	None	Yes	1
13	62	None	Yes	3
13	63	None	Yes	4
14	64	3.0 mg/ml	No	0
14	65	3.0 mg/ml	No	0
14	66	3.0 mg/ml	No	0

* Provided continuously in water in lightproof drinking bottle to provide an estimated total dose of 800 mg/kg/day.

given only valproic acid (Group 14) died during the study. Tissue cysts were structurally normal when viewed as fresh preparations with light microscopy. They were also normal when viewed in stained histological sections. The mean number of tissue cysts in 50 μ l was 1.3 ± 1.3 (range, 0–3) for mice in Group 12 and 2.0 ± 1.8 (range, 0–4) for mice in Group 13. No tissue cysts were seen in the brains of mice in Group 14. There were no significant differences ($P > 0.05$) in tissue cyst counts in mice infected with the ME49 strain of *T. gondii* and treated with valproic acid (Group 12) and those infected but not treated with valproic acid (Group 13).

Valproic acid is active against tachyzoites of *T. gondii* in 2 different cell-culture-based assays (Jones-Brando et al., 2003; Strobl et al., 2007). The studies of Jones-Brando et al. (2003) indicate that valproic acid is active at lower concentrations than reported by Strobl et al. (2007), but both indicate that it is active and the differences are probably due to different test systems used to determine activity.

The results of the present study indicate that valproic acid is not active in preventing acute toxoplasmosis in mice. The highest oral daily dose of 800 mg/kg/day we tested is approaching the oral LD₅₀ dose of this agent in mice (1,098 mg/kg); the highest i.p. dose of valproic acid tested, 600 mg/kg/day, is greater than the LD₅₀ in mice for this route of administration (470 mg/kg) (American Pharmaceutical Partners, Inc., Bedford Labs, Bedford, Ohio). Therefore, further increasing the dose of valproic acid is impractical. In contrast, Webster et al. (2006) demonstrated that oral treatment of chronically *T. gondii*-infected rats with 40 mg/kg/day of valproic acid was beneficial in their study system. Treated *T. gondii*-infected rats retained their innate avoidance of feline smell (cat urine). The mode of action of valproic acid, including which stage of *T. gondii* is affected by valproic acid in the rats, is not known. Because the rats were chronically infected, it is possible that valproic acid acted on the tissue cyst/bradyzoite stages of *T. gondii*. Valproic acid might also influence the bradyzoite-to-tachyzoite or tachyzoite-to-bradyzoite stage conversion. We did not demonstrate a significant ($P > 0.05$) effect of valproic acid on the numbers of tissue cysts in treated mice versus controls. Additional study is needed to examine the activity of valproic acid and similar agents against the tissue cysts/bradyzoites of *T. gondii*.

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Molecular Characterization of *Babesia kiwensis* From the Brown Kiwi (*Apteryx mantelli*)

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ABSTRACT: To further investigate the recently described avian piroplasm, *Babesia kiwensis*, blood samples were collected from 13 wild-caught and 8 zoo-captive brown kiwi (*Apteryx mantelli*) and screened for the presence of piroplasm DNA using a nested-polymerase chain reaction (PCR) targeting the 18S rRNA gene of most members of Piroplasmida. All captive birds gave a negative PCR result, while 12 wild-caught birds were PCR positive. The nearly full-length 18S rRNA gene for *B. kiwensis* was sequenced. Upon phylogenetic analysis, it was found to belong to the babesid

group of piroplasms and was ancestral, yet genetically similar, to the *Babesia canis*-related species. An insight into the current taxonomy of the avian piroplasms is also given. An *Ixodes anatis* tick collected from 1 of the North Island brown kiwi was also screened using PCR and was found to be positive for *B. kiwensis* DNA.

The avian piroplasms (Piroplasmida) remain an understudied group of protozoans. In comparison to many of the mammalian species of