Activity of minocycline against *Toxoplasma gondii* infection in mice

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The chemotherapeutic activity of minocycline, a semi-synthetic tetracycline analogue, was evaluated in a murine model of toxoplasmosis. A lethal acute toxoplasmosis was produced by injecting 10^5 tachyzoites of the RH strain of *Toxoplasma gondii* into the peritoneal cavities of Swiss-Webster mice. When infected mice were treated once daily for 12 days, starting 2 h after challenge, the survival and cure rates were 100% and 40% respectively after minocycline alone (100 mg/kg per day), 0% and 0% after pyrimethamine alone (8·5 mg/kg per day), and 100% and 50% after combination of the two drugs at the same dosages. Absolute survival and cure with minocycline were observed when mice were treated with two daily doses of 100 mg/kg for 12 days. Mice chronically infected with a low virulent strain of *T. gondii* (Me49) showed a significant reduction in the number of brain cysts after three weeks of treatment with 50 mg/kg per day of minocycline. Minocycline serum levels after a single oral administration of 50 mg/kg or 100 mg/kg to normal mice, peaked at 1·8 mg/l and 10 mg/l after 1 h, respectively, and showed an extended half-life.

Introduction

Encephalitis due to *Toxoplasma gondii* has become a frequent infectious complication in patients suffering from the acquired immunodeficiency syndrome (McCabe & Remington, 1988). Treatment with the synergistic combination of pyrimethamine and sulphadiazine (or a trisulphapyrimidine) (Leport *et al.*, 1988) is followed by an initial good response subsequently hampered by a number of side effects (Glatt, Chirgwin & Landesman, 1988), mainly due to the sulphonamide component of the combination. Moreover, this treatment is not able to eradicate the tissue cyst form of the parasite, which is found in the organs of the infected individuals (Remington & Cavanaugh, 1965). Therefore, there is a clear need for the development of new compounds for treating toxoplasmosis.

The in-vivo therapeutic effect of the semi-synthetic tetracycline analogues, doxycycline and minocycline against acute murine infections with *T. gondii* have been previously described (Chang, Comte & Pechère, 1990; Tabbara, Sakuragi & O'Connor, 1982). In the studies reported here, we assessed the activity of minocycline in acute and chronic murine models of toxoplasmosis.

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Materials and methods

Animals

Female Swiss-Webster mice (Madörin AG, Füllinsdorf, Switzerland) at six to eight weeks of age and weighing 25 ± 1 g were used.

Antimicrobial agents

Minocycline hydrochloride was provided in powder form by Laboratoires Lederle, Oullins, France, and prepared each day in sterile distilled water. Pyrimethamine was provided in powder form by F. Hoffmann-La Roche & Cie, S. A., Basel, Switzerland, and was dissolved in 95% ethanol and diluted in sterile distilled water. In studies in which pyrimethamine was used in combination with minocycline the solutions were mixed prior to use.

T. gondii strains

The highly virulent RH strain of *T. gondii* was used for acute infection. Tachyzoites were obtained from the peritoneal cavities of infected mice as described previously (Chang & Pechère, 1987). For chronic infection, the Me49 strain, a low virulent strain, of *T. gondii* was used (kindly provided by Prof. J. S. Remington, CA, USA).

Animals models

Acute toxoplasmosis. Mice were infected intraperitoneally (ip) with 10^5 tachyzoites of the RH strain of *T. gondii*, i.e., 50,000 times the 100% lethal dose (Chang & Pechère, 1987) in 0·5 ml of sterile 0.9% NaCl. Animals were randomly allocated in groups of ten with free access to food and water. At the end of the 30 day study period the surviving mice were sacrificed and autopsied. Peritoneal exudates were examined microscopically (× 400) for the presence of tachyzoites. When parasites could not be seen the brain was homogenized and inoculated into two new mice. The donor was considered 'cured' if the two recipient mice survived 30 days after injection and no *T. gondii* were present at autopsy.

Chronic toxoplasmosis. Mice were infected ip with 20 cysts of the Me49 strain of *T. gondii* in 0·5 ml of sterile 0·9% NaCl. Prior to starting the experiments five mice were randomly assessed for the presence of brain cysts in order to confirm that chronic infection was established. Animals were used four months later. At the end of the 21 day study period the brains of the animals were aseptically removed and 5 ml of sterile 0·9% NaCl added. The organs were successively passaged through 18-G and 20-G needles with a 10-ml plastic syringe to homogenize them. The number of cysts per brain was determined by light microscopy of 25 μl aliquots and scanning the entire brain preparation.

Therapeutic regimens

For acute infection, oral minocycline was started 2 h after ip infection and was given for 12 days. Treatment was administered every 12 h or 24 h. Pyrimethamine alone was administered by gavage, and pyrimethamine was also administered orally when combined with minocycline. For chronic infection, therapy was started four months

after infection and given once daily by gavage for three weeks. The brains were then removed and the number of brain cysts calculated, as previously described. Control groups of infected mice (for acute and chronic infections) received only distilled water and uninfected mice received drug treatment in all investigations.

Histopathology

The organs (liver, spleen, kidneys, bowel, lungs and brain) of untreated and treated acutely infected mice were removed five days after challenge, fixed in 10% buffered formalin and embedded within paraffin. Sections were cut at 5 μm and stained with haematoxylin and eosin, and used for histological analysis. The same analysis was carried out at day 17 and day 30 after challenge in the treated group (no control animals were available by this time). Normal uninfected mice served as negative controls.

Levels of minocycline in serum

Groups of thirty normal fasting (1 h) mice were given by gavage a single dose of 50 or 100 mg of minocycline per kg; and blood samples were collected at 0·5, 1, 2, 4, 7, 10, 13, 16, 20 and 24 h after treatment. Serum levels of minocycline were assessed with a microbiological assay by using *Staphylococcus aureus* ATCC 6538P, as test organism. The elimination rate constant (β) was calculated from the linear regression of serum concentration-versus-time data. Half-life ($T_{1/2}$) was calculated by employing the following equation: $T_{1/2\beta} = \ln2/\beta$.

Statistics

For survival rates statistical analysis was performed by using the chi square test. Other differences between test and control groups were analysed using Student's t-test. A P value of < 0.05 was considered significant.

Results

Acute toxoplasmosis

All infected untreated control mice died from acute toxoplasmosis 5 ± 1 days after challenge (Table I). All minocycline-treated infected mice survived. A dose of 100 mg/kg/day of minocycline given for 12 days protected 100% of the mice ($P < 0.001$, compared with untreated controls) with a cure rate of 40%, according to brain sub-inoculations. When this dose of minocycline was given in combination with pyrimethamine (8·5 mg/kg/day), 100% of animals survived with a cure rate of 50%. Pyrimethamine (8·5 mg/kg/day) alone was ineffective in protecting the animals. Complete protection (100%) with total cure was obtained with minocycline in 100 mg/kg doses given every 12 h.

Histopathological studies were performed with a series of infected animals treated with 200 mg/kg/day of minocycline (Figure 1). Five days after challenge, infected control animals showed marked inflammation and extensive necrosis in the liver, spleen, kidney and lungs; the treated mice showed less inflammation and necrosis was not observed. Seventeen days after challenge, the treated mice showed even less

Table I. Therapeutic effects obtained with minocycline and pyrimethamine on murine acute toxoplasmosis

Drug (dose (mg/kg per day))	Time to death for 50% of mice (days)	No. of survivors/ no. of mice (%)	No. of survivors cured (%)
None	5 ± 1	0/30 (0)	
Minocycline			
25	9	3/10 (30)	0/3 (0)
50	15	5/10 (50)	2/5 (40)
100		10/10 (100)	4/10 (40)
200		10/10 (100)	10/10 (100)
Minocycline (100) + pyrimethamine (8·5)	·	10/10 (100)	5/10 (50)
Pyrimethamine (8·5)	8	0/10 (0)	

inflammation in the organs examined, and by day 30 inflammation had completely resolved.

Chronic toxoplasmosis

No spontaneous death was observed during the entire period of study. The number of brain cysts in the mice treated with minocycline at a dose of 50 mg/kg/day for 21 days was significantly lower ($P < 0.02$) as compared to the untreated control mice (Table II).

Levels of minocycline in the serum of mice

Minocycline attained peak serum concentrations of 1·8 and 10 mg/l 1 h after a single dose of 50 or 100 mg/kg, respectively. The calculated serum half-lives were 5·4 and
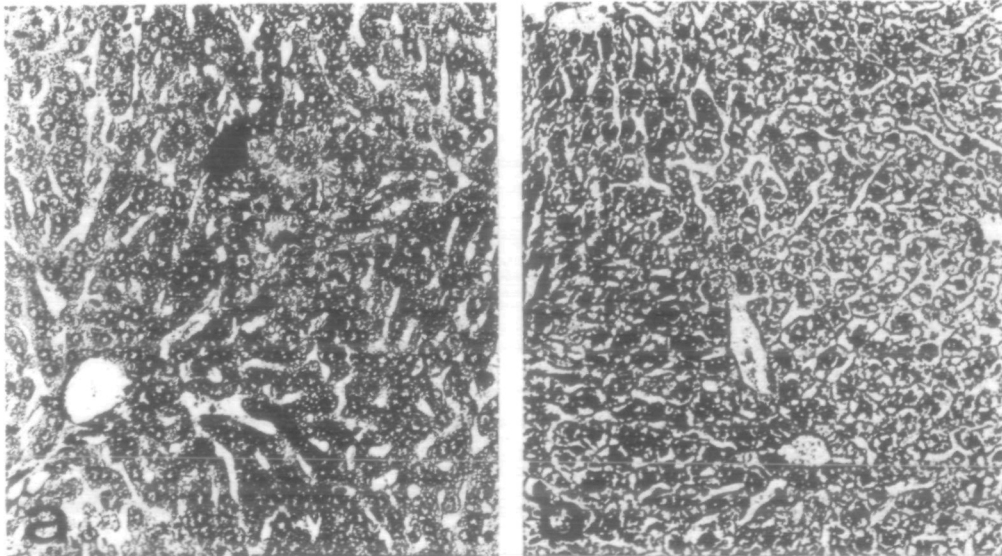

Figure 1. Histological sections of the livers of control (a) and treated (b) mice (200 mg/kg/day) five days after infection with 10^5 tachyzoites of the RH strain of *T. gondii*. Arrow indicates focus of necrosis. Magnification × 160.

Table II. Effect of minocycline on the number of brain cysts in
T. gondii-infected mice

Drug dose (mg/kg per day))	Number of mice	Mean brain cysts/ mice ±SEM[a]
None	10	981±78
Minocycline (50)	10	660±82[b]

[a]The mean number ±s.e.m. of brain cysts at the time of starting
experiments was 390±28 (for five mice).
[b]$P < 0.02$, as compared with control mice.

8·2 h after 50 and 100 mg/kg, respectively. Presence of the drug was still detected 16 h
after administration of the higher dose (Figure 2).

Discussion

Minocycline protected and cured mice lethally infected with a high inoculum of the
virulent RH strain of *T. gondii*. The potent effect of minocycline on *T. gondii* was
further documented by histological studies which showed that tissue damage seen in the
untreated controls was completely avoided in the treated mice. This confirms and
extends the findings of Tabbara *et al.* (1982) who were able to protect 71% of mice
infected with 7×10^5 *T. gondii* RH strain parasites. The reason these authors were
unable to protect all the mice in their studies seems to be due to the extremely high
parasite inoculum used which was seven times more than in our study. The better cure
rate obtained with the twice-a-day dosing compared with the once-a-day regimen may
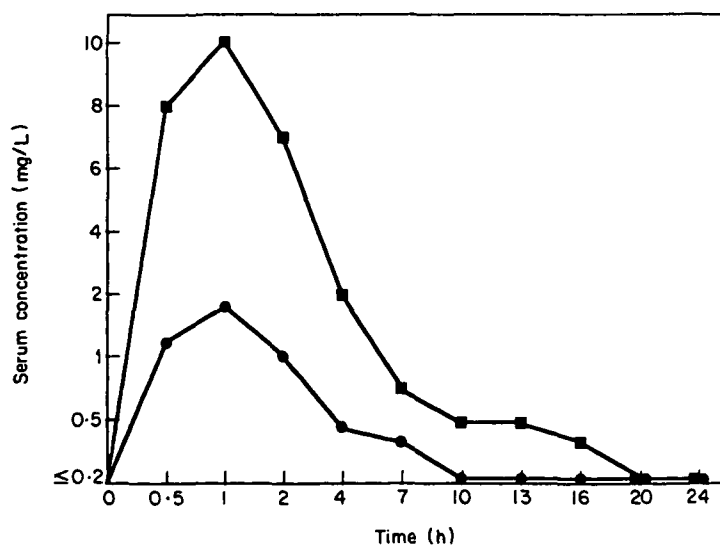well be related to a more sustained antibiotic coverage as shown by the serum

Figure 2. Mean serum pharmacokinetics of minocycline in normal Swiss Webster mice after single peroral
doses of 50 mg/kg (●) and 100 mg/kg (■). There were three mice per each data point.

pharmacokinetic curves. A close comparison between murine and human pharmacokinetics is not feasible from the available data. However, minocycline serum concentrations associated with full protection of the mice were in the range of serum concentrations achievable in humans where peak levels have been found at 8·75 mg/l after a single iv infusion of 100 mg of minocycline (Welling *et al.*, 1975). Pyrimethamine at 8·5 mg/kg did not enhance significantly the cure rate obtained with 100 mg/kg per day of minocycline. This observation may suggest either a lack of synergism or additive effect between the two compounds, or insufficient penetration of pyrimethamine at the dose tested into the brains of the infected mice.

In our chronic infection model, fewer brain cysts were counted in the minocycline treated than untreated animals, suggesting that minocycline had reached brain tissue. Since, in the treated animals, less cysts were counted at initiation of therapy than after therapy we assume that minocycline had an inhibitory preventive effect rather than an actual toxoplasmacidal therapeutic effect. Minocycline is highly lipophilic and diffuses into the CSF in significant amounts with concentrations that vary from 15%–65% of the blood concentrations (McDonald *et al.*, 1973). The concentration in human brain tissues of minocycline after oral or iv administrations is not known, but minocycline has been found to achieve a concentration of about 2·5 μg/g in the brains of dogs after an iv infusion of a loading dose of 5 mg/kg over 10 to 30 min followed by a continuous infusion of 1 mg/kg per h during 3 h (Barza *et al.*, 1975).

The mechanism of action of minocycline on *T. gondii* is unclear at this time. As suggested by previous work from our laboratory, some inhibitors of protein synthesis (i.e. macrolides and tetracycline analogues) possess different degrees of anti-Toxoplasma activity (Chang & Pechère, 1987, 1988; Chang, Rudareanu & Pechère, 1988; Chang *et al.*, 1990). However, amongst those drugs, and on a weight-to-weight basis, minocycline appears to be the most active therapeutic agent in the mouse model of acute toxoplasmosis.

In conclusion, our studies demonstrate the potent activity of minocycline in murine infections caused by *T. gondii*. The possibility of using minocycline in the treatment of toxoplasmic infections, including encephalitis, in nonpregnant patients suffering from AIDS, deserves consideration.

Acknowledgements

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