6. Summary

Primary infection with *Toxoplasma gondii* usually does not cause clinical symptoms. Infection is characterized by persistence of the parasite in cysts in the central nervous system. In immunocompromized hosts (i.e. AIDS- and transplanted patients) reactivation may occur and results in development of toxoplasmic encephalitis. Reactivation takes a lethal course if left untreated. Therapy comprises a 4-week course of acute treatment and lifelong maintenance treatment. Standard therapy regimens often cause allergy or have toxic side effects. Alternative therapy regimens show low bioavailability and/or do only poorly penetrate the blood-brain-barrier (BBB). Antiparasitic drugs prepared as nanosuspenions with modified surfaces (i.e. tween 80-coated) allow targeting of the central nervous system.

To investigate the passage of antiparasitic nanosuspensions through the BBB, we established a co-culture transwell model of the BBB using primary rat brain endothelial cells and primary rat astrocytes. This co-culture model was superior to the commonly applied models using mouse or rat brain endothelial cell lines. We observed increased electrical resistance (TEER of up to 350 Ω x cm^2) as well as low paracellular permeability using sodium fluorescein as a marker. However, the passage of tween 80-coated nanosuspensions through the transwell system, was not dose dependent, most likely caised by aggregation of nanosuspensions and adherence to the polycarbonate filters was observed. To study the interaction of *T. gondii* tachyzoites with the BBB, we followed the infection of primary brain endothelial cells by *T. gondii* using laser-scanning microscopy. We observed rapid entry of the parasites into brain endothelial cells. In parasite-cell-ratios of >1:1, parasites showed dose-dependent cytotoxic effects on endothelial cells.

Tween 80-coated nanosuspensions of the lipophilic hydronaphthochinone atovaquone (showing excellent in-vitro activity against *T. gondii*) were prepared to investigate targeting of the brain in-vivo. Dosage, time, and frequency of i.v.-administration of atovaquone nanosuspensions for acute treatment were optimized in a murine model of reactivated toxoplasmosis.

In addition, we expanded the murine model of acute therapy to study the therapeutic

efficacy of drugs in maintenance therapy. Oral administration of atovaquone suspensions showed superior therapeutic efficacy compared to standard therapy using the combination of pyrimethamine and sulfadiazine. Time to death was prolonged and histological signs of toxoplasmic encephalitis were absent in mice orally treated with atovaquone. High concentrations of atovaquone were detectable in sera, liver, and lungs of mice using HPLC whereas in brains of mice atovaquone was only detectable by highly sensitive mass spectrometry.

Results of the present study will allow further studies to follow passage of antiparasitic drugs through the BBB. The co-culture model also allows to track transmigration of the parasite through the BBB. The murine model of reactivated toxoplasmosis closely mimics the clinical situation in immunocompromized hosts and therefore allows testing of antiparasitic drugs for acute and maintenance therapy in-vivo. Atovaquone should be further tested in clinical trials for acute and maintenance treatment of toxoplasmic encephalitis.