Sulfadiazine resistance in *Toxoplasma gondii*: transcriptomic approach

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26th ECCMID
Amsterdam, Netherlands
9 – 12 April 2016

Background

Toxoplasma gondii, the causative agent of toxoplasmosis, is a protozoan parasite that is estimated to infect more than one-third of the world's human population. Its life cycle comprises a series of five developmental stages in the intestinal epithelium of the feline definitive host and three stages relevant to infection in intermediate hosts which include all other species of mammals. These stages are sporozoites (contained in oocysts), and the obligate intracellular stages tachyzoites, and bradyzoites.

The principal drugs known as active against *T. gondii* are divided into two major groups: the inhibitors of folic acid synthesis and the macrolides. These drugs are primarily active against the tachyzoites and have no effect on cysts.

The inhibitors of folic acid synthesis include dihydrofolate reductase (DHFR) inhibitors (pyrimethamine and trimethoprim), and dihydropteroate synthase (DHPS) inhibitors (sulfonamides). These two anti-*Toxoplasma* drugs are used in combination because of a synergistic effect on two key enzymes of folic acid metabolism, DHPS and DHFR. These drugs act together by inhibiting the folic acid synthesis in *T. gondii*, but also that of its host. As a consequence, they have a powerful anti-parasitic effect (changes of purine synthesis and parasitic division), which is associated with adverse haematological events (neutropenia, thrombocytopenia). These drugs diffuse well into the organism and cross the placental barrier. The most often prescribed active associations are pyrimethamine-(sulfadiazine or sulfadoxine), and trimethoprim-sulfamethoxazole. These associations (except the pyrimethamine-sulfadoxine association) are used for the treatment of severe toxoplasmosis in immunocompromised patients. The pyrimethamine-(sulfadiazine or sulfadoxine) association is used in congenital toxoplasmosis (prenatal and postnatal treatment). As indicated above, in order to limit adverse haematological events, these treatments are administered with folinic acid.

In clinical practice, therapeutic failures and relapses have been observed. The understanding of the failure mechanisms against the main active drugs on *T. gondii* is essential because there are currently few effective and validated therapeutic alternatives. Considering a drug's action mechanism, and in analogy to the other protozoa, the existence of a sensitivity change for drugs and/or the development of drug resistance could be feared.

Objectives

In order to better understand the mechanisms of resistance to sulfadiazine, we have analysed the expression levels of 7 folate transporter genes [1] (Fig. 1), 11 ABC transporter genes [2], and 14 enzyme genes which are involved in folic acid synthesis. This study by real time RT-PCR focused on sensitive strains (RH and ME-49), natural resistant strains (TgH 32006 and TgA 103001) [3], and induced resistant strains (RH-R^{SDZ} and ME-49-R^{SDZ}) [4].

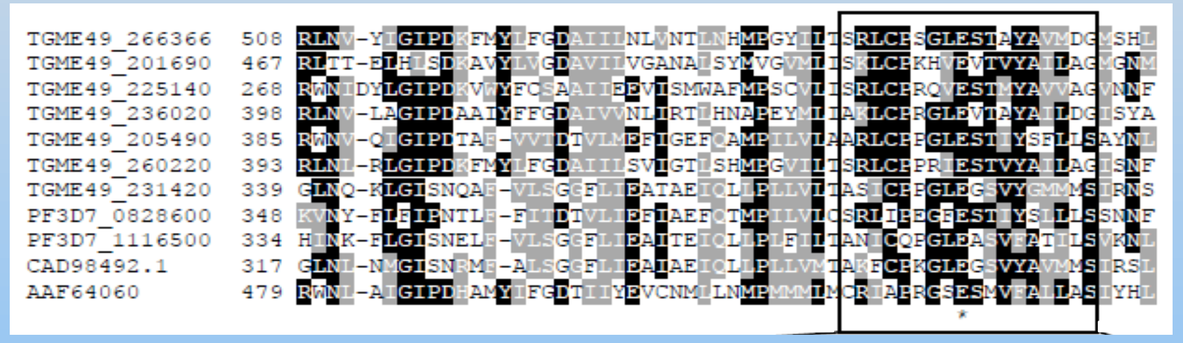

Fig. 1. Multiple alignment of folate-biopterin transporters (FBT) relevant to this work

The depth of shading represents degrees of similarity. A (*) symbol indicates residues identical in all sequences. The signature sequence for the FBT family (black box) has been identified in all sequences.

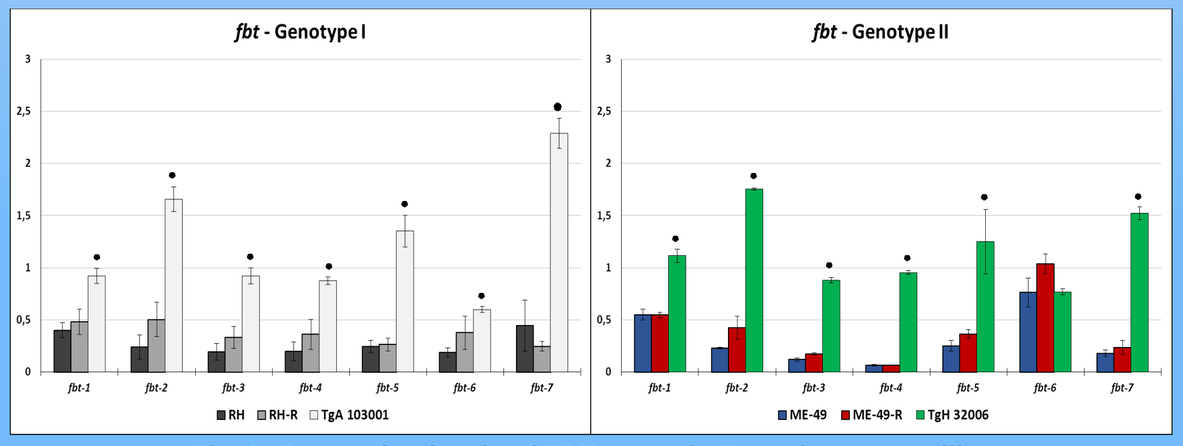

Fig. 2. Expression levels of FBT genes in Toxoplasma gondii

Results - Discussion

The transcriptomic analysis of various *T. gondii* strains has highlighted in resistant strains an overexpression of folate transporter genes (Fig. 2), one ABC transporter gene (data not shown), and 2 enzyme genes which are involved in folic acid synthesis (data not shown). Overexpression of these genes would allow the survival of parasite, by the internalization and the sequestration of folate, and the efflux of sulfadiazine. Furthermore, the potential involvement of folate transporters in drug resistance could, in the long term, raise the question about the systematic administration of folinic acid, which could potentially enhance the parasite's survival.

References

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[2] Sauvage (2004) Mol. Biochem. Parasitol. 134, 89-95.
[3] Meneceur (2008) Antimicrob. Agents Chemother. 52, 1269-77.
[4] Doliwa (2013) Experimental Parasitol. 133, 131-36.