**In vitro efficacy of nitro- and bromo-thiazolyl-salicylamide compounds (thiazolides) against Besnoitia besnoiti infection in Vero cells**

SUMMARY

Nitazoxanide (NTZ) and its deacetylated metabolite tizoxanide (TIZ) exhibit considerable in vitro activity against Besnoitia besnoiti tachyzoites grown in Vero cells. Real-time-PCR was used to assess B. besnoiti tachyzoite adhesion, invasion, and intracellular proliferation in vitro. A number of NTZ-derivatives, including Rm4822 and Rm4803, were generated, in which the thiazole-ring-associated nitro-group was replaced by a bromo-moiety. We here show that replacement of the nitro-group on the thiazole ring with a bromo (as it occurs in Rm4822) does not impair the efficacy of the drug, but methylation of the salicylate ring at the ortho-position in a bromo-derivative (Rm4803) results in complete abrogation of the antiparasitic activity. Treatment of extracellular B. besnoiti tachyzoites with NTZ has an inhibitory effect on host cell invasion, while treatments with TIZ, Rm4822 do not.TEMdemonstrates that the effects of Rm4822 treatment upon the parasites are similar to the damage induced by NTZ. This includes increased vacuolization of the parasite cytoplasm, and loss of the structural integrity of the parasitophorous vacuole and its membrane. Thus, Rm4822, due to the absence of a potentially mutagenic nitro-group, may represent an important potential addition to the anti-parasitic arsenal for food animal production, especially in cattle.

Key words: thiazolides, Besnoitia besnoiti, in vitro drug treatment, besnoitiosis.