Conference presentation

Effects of homeopathic medicines on macrophage – *E. cuniculi* interaction *in vitro*

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Abstract

Encephalitozoon cuniculi (*E. cuniculi*) is a fungus that behaves as an intracellular parasite infecting different types of cells. In rabbits and immunosuppressed animals of other species, including humans, it parasites neural tissues causing a highly characteristic neurological syndrome, for which repertory analysis indicates *Phosphorus*. Successful treatment of 7 naturally infected rabbits with *Phosphorus* confirmed that this medicine probably is the remedy of the 'epidemic genius' of microsporidiosis. In the present study, an *in vitro* model was used to evidence the intracellular relationship between macrophages and *E. cuniculi* after treatment of co-cultures with different potencies of *Phosphorus*. RAW 254.7 macrophages were co-cultivated with *E. cuniculi* and treated with *Phosphorus* 6cH, 30cH and 200cH. Controls were untreated co-cultures and cultures treated with vehicle (0.06% final alcohol concentration). Phagocytosis and lysosome activity were after 1 and 24 hours of incubation by means of the calcofluor and acridine orange staining methods, followed by automatic image analysis (Metamorph®). Cytokine production was assessed with the MAGPIX®-Luminex system. The vehicle increased IL-6, MCP-1 and MIP1 production ($p<0.05$) and reduced the number of phagocytized parasites ($p<0.0001$) after 1-hour incubation, but no effect was detected after 24 hours of treatment. *Phosphorus* 6cH increased lysosome activity after 1- and 24-hour incubation and reduced the number of phagocytized parasites after 24 hours ($p<0.05$). *Phosphorus* 30cH increased lysosome activity after 1-hour incubation, followed by reduction of parasite internalization ($p<0.001$), and increased MCP-1 production ($p<0.05$) after 24 hours compared to both controls. The 200cH potency increased lysosome activity at 1 and 24 hours ($p<0.05$), together with reduction of internalized parasites ($p<0.01$) and increase of MCP-1 ($p<0.05$) compared to both untreated cells and cells treated with vehicle. The ultrastructural features of internalized parasites following treatment with *Phosphorus* 200cH is still under investigation. The results show that treatment of co-cultures with *Phosphorus* 200cH caused significant changes in the macrophage – *E. cuniculi* interaction, which might be involved in the clinical improvement of sick animals treated with this medicine.

Keywords: *Encephalitozoon cuniculi*, macrophages, *Phosphorus*, *in vitro* models

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