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 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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