Evaluation of biological activities of highly diluted nucleotide sequences by using cellular models

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ABSTRACT

Background: highly diluted specific nucleic acids (SNA®), designed to modulate viral and cytokine genes expression, are currently used in Micro-Immunotherapy to treat viral infections and immune disorders. Although some preliminary studies have showed clinical benefit of these homeopathic preparations [1], no experimental data are available to explain their mechanism of action.

Aims: to investigate the *in vitro* effect of two sets of highly diluted (HD) SNA targeting i) latent/lytic Epstein-Barr virus (SNA EBV) and ii) TNF- α and its receptor p55 involved in rheumatoid arthritis (SNA RA) on cellular models.

Methodology: serial homeopathic dilutions of SNA EBV and SNA RA (15cH-18cH) were tested on a EBV-positive B-lymphoblastoid (B95-8) and on a LPS-stimulated macrophage (THP1) cell lines respectively, in comparison with agitated/diluted water and scramble DNA sequences prepared in the same conditions (negative controls). For B95-8 proliferative model, high mobility group box 1 protein (HMGB1) was used as reference. Analyzed biological parameters on B95-8 were i) cell proliferation measured after 24 and 48h of incubation with HD SNA and ii) expression of the EBV ZEBRA protein in response to TGF- β by Western-blotting (T+24h). For THP1 model, TNF- α synthesis and release were determined by RT-qPCR and ELISA (protein), after stimulation by LPS (1 μ g/ml) and HD SNA co-administration.

Results: we demonstrated that HD SNA RA significantly down-regulated TNF- α synthesis and release. This biological activity was showed to be specific (no effect of HD scramble SNA) and related to the level of dilution (maximal effect with higher dilutions). Unexpectedly, a biological effect of agitated/diluted water was also detected in both cellular models. For B95-8 model, this effect resulted in a significant decrease of B95-8 proliferation (comparable to the HMGB1 reference) and an inhibition of ZEBRA expression. Similarly, a reproducible stimulation effect of HD water was obtained in the LPS-stimulated THP1 model.

Conclusions: these findings indicate that highly diluted SNA RA can regulate TNF- α synthesis and release by LPS-stimulated THP1 and support the hypothesis that these homeopathic preparations may act in modulating mRNA expression of the targeted genes. This *in vitro* work underlines the potential effect of agitated water in context of cellular models for testing biological properties of HD.

Keywords: Specific Nucleic Acids (SNA®); Cellular models; Epstein-Barr virus, Tumor Necrosis Factor- α , Rheumatoid arthritis

References:

[1] Jenaer M, Henry MF, Garcia A, Marichal B. Evaluation of 2LHERP in preventing recurrences of genital herpes. *Br Homeopath J*. 2000 Oct;89(4):174-7.

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Conflict of interest: Ghada Alsaleh, Chloé Borde, Dominique Wachsmann, and Vincent Maréchal declare there is no conflict of interest. Etienne Capieaux and Pierre Dorfman are respectively Scientific Director and Medical Director at Labo'Life.

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