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ABSTRACTS - ORAL PRESENTATIONS

In vitro testing of highly diluted cytokines and specific nucleotide acid sequences applied in micro-immunotherapy for rheumatoid arthritis

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Background: TNF-α and IL-6 are key inflammatory factors in rheumatoid arthritis (RA) and constitute targets for the development of anti-inflammatory drugs. Rather than apply antagonist strategies, the micro-immunotherapy approach is based on the use of very low doses and highly diluted cytokines and specific nucleotide acid sequences (SNA®) which, administered sequentially, are intended to reduce synovial inflammation and to regulate auto-immune disorders associated with RA.

Objectives: the aim of these *in vitro* studies was double: i) assess on various cellular models the biological activities of serial homeopathic dilutions of cytokines and SNA developed for a new Micro-Immunotherapy medication (2L®PR) and ii) investigate their mechanism of action by using biomolecular tools.

Methods: a first set of experiments was performed on human fibroblast-like synoviocytes (FLS) isolated from RA patients and cultured in standardized conditions. Different protocols of treatment were applied to examine the potential anti-inflammatory effect of major cytokines (IL-1, IL-2, IL-6, IL-10, IFN-γ, TNF-α) administered in a large range of dilutions (3CH to 27CH). Homeopathic solutions were tested alone or in association on FLS activated with various concentrations of TNF-α (0.1, 1 and 5 ng/ml). Preliminary tests were carried out on non-activated FLS. IL-6 release was determined in cell supernatants by ELISA. In addition, the anti-inflammatory effect of TNF-alpha 5CH formulated in homeopathic pellets was controlled on this FLS model. In a second set of experiments, high dilutions (HD) of SNA sequences designed to target the gene of two major proteins involved in RA (TNF-α and its receptor p55) were investigated on a LPS-stimulated macrophage (THP1) model. TNF-α synthesis and release were determined by RT-PCR (mRNA) and ELISA (protein), after stimulation by LPS (1μg/ml).

Results: in the first set of experiments, we observed that priming of cells with TNF-α and IL-6 dilutions down-regulated IL-6 release by TNF-α activated FLS. The same result was obtained with pellets of TNF-α 5CH. This effect was not obtained with other major cytokines such as IL-1, IL-Ra, IL-2, IL-10, and IFN-γ. In the second set of experiments, we demonstrated that HD of both SNA 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 reproducible stimulation effect of HD water was obtained in the LPS-stimulated THP1 model. This biological activity of agitated water (negative control) was not detected in TNF-α activated FLS model.

Conclusions: these findings indicate that homeopathic dilutions of TNF-α and IL-6 can regulate IL-6 release by synoviocytes and that highly diluted SNA RA can regulate TNF-α synthesis and release by LPS-stimulated THP1. This exploratory work supports the hypothesis that micro-immunotherapy may represent an alternative therapeutic approach for RA and that high dilutions act in modulating mRNA expression of the targeted genes.

Keywords: Specific Nucleic Acids (SNA®); Cytokines; Micro-Immunotherapy; Cellular models; Rheumatoid arthritis

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Homeopathic basic research: state of research and quests for the future

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Homeopathy relies on two basic tenets: the simile principle and the potentisation procedure. The validity of these presumptions is being questioned since there seems to be no obvious scientific basis supporting justifiable application in pharmacy and medicine. Nevertheless, homeopathy is being practised and many patients as well as practitioners are quite satisfied with clinical outcome in daily practice. However, the lacking understanding does lead not only to problems with legal recognition, integration into public healthcare and reimbursement by health insurances, but