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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

also hampers further development and optimisation of homeopathic therapy. Therefore, development of a deeper understanding of the two basic tenets of homeopathy is of ultimate importance.

Only few basic research projects seem to have been performed to investigate the simile principle. The fundamental pioneering work of van Wijk and Wiegant so far has not been taken up by any other research team. Determination of the areas of applicability of the simile principle is an important task, as is the elucidation of the mode of action.

Comparably more research has been carried out to investigate the potentisation procedure. However, I currently do not know any laboratory model that reproducibly yields specific effects of highly diluted homeopathic potencies in different laboratories, and I do not know any theory that would satisfyingly explain any such specific effects of ultramolecular potencies. Thus I think that the following two main topics have to be addressed in the next years: development of optimal laboratory models to identify specificity and reproducibility of homeopathic effects, and identification of the long-sought-for mode of action of highly diluted potencies.

Are there any experimental laboratory systems that reliably yield reproducible evidence for specific effects of homeopathic potencies? To resolve this question, it will be necessary to investigate various model systems in parallel in different laboratories to determine any necessary and sufficient conditions for successful reproducibility; until now, according to my knowledge, corresponding parameters could be identified for three model systems only. Optimisation of the laboratory models does involve the choice of the test organism in a defined physiological state, an adapted potentised substance in an adequate potency level applied in an optimal route and dosage as well as optimal outcome measures. Furthermore, it will be necessary to develop model systems that not only demonstrate empirical effects of single homeopathic remedies, but also differentiate effects of different potentised substances. Thus, model systems have to be simple and cost-effective to enable easy implementation in other laboratories, and to allow multiple parameters to be tested in parallel (e.g. different substances and/or potency levels). Finally, stability of any experimental system used must be demonstrated by systematic negative control (SNC) experiments on a routine basis.

Identification of the mode of action of highly diluted homeopathic remedies is the ultimate goal of homeopathic basic research. This involves determination of the general type of interaction present between homeopathic potency and test organism: local material-like, force-like, or nonlocal entanglement-like. This not only implies precise investigations of homeopathic preparations by sophisticated physicochemical methods, but also experimental approaches to test Hahnemann's premise of force-like effects of homeopathic potencies. Furthermore, the general nature of the effects of homeopathic potencies has to determined: reproducibly deterministic, chaotic or inherently indeterministic. Solid experimental data regarding these questions will enable development of a precise theoretical framework, ultimately resulting in a thorough understanding of homeopathic effects.

Integrative nanomedicine: homeopathic remedies as source and silica nanoparticles acting as danger signals for nonlinear complex adaptive systems

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The alleged "implausibility" of homeopathic medicines is a foundation for attacks on homeopathy. Skeptics insist that homeopathic medicines are too dilute to contain any residual material from their mineral, plant, or animal sources or exert effects. Nonetheless, multiple studies on cells, animals, plants, and human subjects have demonstrated biological effects of remedies.

Research laboratories have shown that 6 different metal remedies and 3 different plant remedies contain persistent remedy source nanoparticles (NPs) from low to high potencies beyond Avogadro's number for bulk form materials. Multiple laboratories also have documented the ability of succussion in glass containers to release measurable amounts of silicon and silica into solution. Chikramane et al. (2012) showed that succussion can generate heterogeneous accumulation and layers of remedy source nanoparticles that lead to physical transfer carryover of nanoparticles from container to container during the "serial dilution" procedures, even though bulk form source materials may be diluted away. In addition, Das et al. (2013) demonstrated that homeopathic plant mother tinctures can biosynthesize silver nanoparticles from precursors, just as plant extracts can biosynthesize silica nanoparticles from silica precursors.

Nanoparticles could explain many puzzling observations and variability from study to study in homeopathic research. Elia et al. have found aging-related effects in homeopathically-prepared remedies in terms of heat release and electrical conductivity changes after storage — observations that overlap nanoparticle phenomena of aging and Ostwald ripening. Some homeopathically-prepared materials, e.g., specific bacteria nanoparticulates, also emit detectable electromagnetic signals after certain dilution-succussion processes. Certain spectroscopy studies showed unique patterns for homeopathically-prepared remedies compared with control solvents (succussed and nonsuccussed). Some investigators have also interpreted findings from proving studies as indications of quantum mechanical properties of remedies. NPs have enhanced bioavailability, adsorptive capabilities, adjuvant reactivity, electromagnetic, optical, thermal, biochemical, and quantum properties compared with their bulk forms.