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ORIGINAL PAPER

Metal nanoparticle induced hormetic activation: a novel mechanism of homeopathic medicines

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Background: **High-potency homeopathic remedies, 30c and 200c have enormous dilution factors of 10^{60} and 10^{400} respectively. Therefore, the presence of physical entities in them is inconceivable. As a result, their efficacy is highly debated and often dismissed as a placebo. Despite several hypotheses postulated to explain the claimed homeopathic efficacy, none have satisfactorily answered the qualms of the sceptics. Against all beliefs and principles of conventional dilution, we have shown that nanoparticles (NPs) of the starting metals are unequivocally found in the 30c and 200c remedies at concentrations of a few pg/ml. In this paper, our aim was to answer the important question of whether such negligible metal concentrations elicit a biological response.**

Methods: **Metal-based homeopathic medicines (30c and 200c) were analysed at doses between 0.003%v/v and 10%v/v in *in-vitro* HepG2 cell-line. Upon treatment, cell response was estimated by MTT assay, FACS and total intracellular protein. Experiments were performed to discern whether the hormesis was a cell-activation or a proliferation effect.**

Results: **Remedies at doses containing a few femtograms/ml levels of the starting metals induced a proliferation-independent hormetic activation by increasing the intracellular protein synthesis. The metal concentrations (at fg/ml) were a billion-fold lower than the studies with synthetic NPs (at μg/ml). Further, we also highlight a few plausible mechanisms initiating a hormetic response at a billion-fold lower dose.**

Conclusions: **Hormetic activation has been shown for the first time with standard homeopathic high-potency remedies. These findings would have a profound effect in understanding these extreme dilutions from a biological perspective.** *Homeopathy* (2017) ■, 1–10.

Keywords: Homeopathy; Hormesis; Nanoparticles

Introduction

Homeopathy today has global prevalence and is used by millions worldwide. Its basic tenet, the 'Law of Potentization', perceives that any substance (e.g. heavy metals, toxins, poisons, etc.) when diluted numerous times with vigorous shaking (succussion) at each dilution step imparts potent medicinal properties to these ultra-high dilutions. However, despite the numerous positive claims worldwide, a major drawback arising through this process is the colossal dilution factors (30c: 10^{60} and 200c: 10^{400}), often way

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beyond the Avogadro's number (6.023×10^{23} molecules/mole). Therefore, perceiving the presence of even remnants of the starting physical entities in these super-Avogadro dilutions is extremely difficult and often considered to be a placebo effect. Though attempts to address this situation have been made through several theories,[1–7] a major stumbling block has been a dearth of theories of physical origin (except Silica hypothesis)[8] combined with the conjectural nature and insufficient validation of the postulated hypotheses. These together have therefore drawn severe criticism from the scientific community.

However, contrary to all conventional dilution principles and existing beliefs, nanoparticles (NPs) of the respective starting metals at a few picograms/ml (pg/ml) levels have been explicitly shown to exist in high potency (30c and 200c) metal-based homeopathic medicines, despite the gargantuan dilutions.[9] The NPs were found to be retained as a result of the manufacturing processes involved. The succussion process aided particle levitation forming a surface-enriched particle monolayer that was effectively transferred through the serial dilutions forming an asymptote below a certain metal concentration.[10]

On the basis of our discovery of NPs of the starting metals in these extreme dilutions, an obvious question that warrants an answer from a biological standpoint is "*Whether these minuscule metal concentrations in homeopathic remedies have the ability to elicit biological effects upon administration?*" This paper addresses this question. We show that a universal biphasic dose phenomenon known as 'Hormesis' applies to homeopathic medicines and can be the plausible mode of action.

Hormesis is a cellular response characterized by a high-dose inhibition and low-dose cellular activation.[11–15] Hormetic low-dose stimulation has been shown to occur in various *in-vitro* biological models with numerous chemicals, both natural and synthetically manufactured. Notable among these, have been the highly-potent toxins and heavy metals.[16,17] Studies on the hormetic effects of various substances have pointed towards an increased ability of cells to withstand higher levels of stressors upon prior low-level stress exposure. Thus, hormesis gives the cells an ability to correct the homeostatic disturbance and preserve intracellular conditions.[18–21] These findings have been corroborated with the biochemical analyses which have unequivocally shown an activation of various cell survival mechanisms, including the antioxidant enzymes[22] and the mitogen-activated protein kinase (MAPK) pathways.[13]

In this article, we have answered the above question of a potential biological response of minuscule metal concentrations in studies on HepG2 human hepatocarcinoma cell-line. The five metal-based homeopathic medicines [*Aurum met* (*Au met*), *Plumbum met* (*Pb met*), *Stannum met* (*Sn met*), *Zincum met* (*Zn met*) and *Argentum met* (*Ag met*)] were obtained commercially from authorized distributors of SBL, a leading homeopathic manufacturer in India. Two potencies, 30c and 200c of *Au met* and *Pb met* were studied, whereas, three potencies – 6c, 30c and 200c were used for other medicines. Upon treatment with extremely low-doses of the metal-based homeopathic medicines effectively containing metals at a few femtograms/ml (fg/ml) concentrations, a proliferation-independent hormetic stimulation was shown using MTT cell activation assay, Fluorescence-assisted cell sorting (FACS) assays and intracellular protein estimation. This is fascinating because even though the two high-dilution phenomena i.e. hormesis and homeopathy have been invoked together earlier,[23–28] for the first time such hormetic activation has been shown in standard homeopathic remedies. We also discuss a few plausible mechanisms to explain the observed stimulation with infinitesimal metal doses in the homeopathic remedies, a billion-fold lower than the effect observed using synthetic NPs.

Materials and methods

Cell cultures and treatment

The HepG2 cell-line was obtained from the National Centre for Cell Sciences (NCCS, Pune, India) and cultured in 25 cm^2 flasks in Dulbecco's modified eagle medium (DMEM; Gibco-Invitrogen, USA) supplemented with 10% heat-inactivated fetal bovine serum (FBS; Gibco-Invitrogen, USA) and 50 U/ml of penicillin-streptomycin (Himedia, India) in a CO_2-incubator maintained at 37°C in a humidified atmosphere of 5% CO_2. The cells were sub-cultured twice a week. Cells between passages 25–80 were used for the studies.

MTT cell activation assay

MTT ([(3-[4,5-dimethyl-2-thiazol-2-yl]-2,5-diphenyl-tetrazolium bromide)]; Sigma Chemicals, USA) assay was used to measure the relative cellular activity after treatment with the metal-based homeopathic medicines. In the concentration-dependent assays, *Zn met* and *Ag met* were analyzed at the following concentrations: 0.003%v/v, 0.01%v/v, 0.03%v/v, 0.1%v/v, 0.3%v/v, 3%v/v and 10% v/v, whereas *Sn met*, *Au met* and *Pb met* were analyzed from 0.01%v/v to 10%v/v. The concentrations, measured on per volume basis of medium in the wells, were decided so as to get a complete hormetic curve for each medicine. HepG2 cells were seeded in DMEM at 0.75×10^5 cells/ml density in a 24-well plate (BD Biosciences, USA) and incubated overnight. On the following day, 1 ml of fresh medium pre-mixed with the measured quantities of the homeopathic medicines or 90%v/v ethanol (EtOH; HPLC Grade – Commercial Alcohols Inc., Canada) depending upon on the group assigned to each well was added. The plates were further incubated for 48 h after which 100 μl of 0.22 μm filtered solution of MTT (5 mg/ml) was added. Following an additional incubation for 4 h at 37°C to allow formazan crystal formation, 1 ml of dimethyl sulphoxide (DMSO; Merck Speciality Chemicals, India) was added to each well and the crystals solubilised. The reduction of the yellow-coloured MTT to a purple-coloured formazan product by the mitochondrial dehydrogenases of the viable cells was measured as change in the optical density, on an ELISA plate reader at 550 nm. All the experiments

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