Homeopathy (2017) ■, 1–11
© 2017 The Faculty of Homeopathy. Published by Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.homp.2017.02.004, available online at http://www.sciencedirect.com

Effect of *Zincum metallicum* on cell models I

Jenifer Pendiuk Gonçalves, Maria Luiza Ferreira dos Santos, Gustavo Rodrigues Rossi, Viviana Stephanie Costa Gagosian and Carolina Camargo de Oliveira*

Laboratory of Inflammatory and Neoplasic Cells, Cell Biology Department of Biological Sciences, Universidade Federal do Paraná, Av Cel Francisco H dos Santos, s/n, CEP 81530-980, Curitiba, PR, Brazil

Introduction: Zinc is an essential trace elements necessary for life maintenance. Traditional and complementary medicines use zinc-based formulations to treat different classes of diseases. Basic research on homeopathic preparations of zinc are rare, there are a few published clinical cases describing its effects on patients. The use of cell-based models in drug screening is a reliable source evidence.

Methods: We sought to investigate experimental end-points using cell-based models to determine the effects of dilutions of ***Zincum metallicum*** prepared according to the Brazilian Homeopathic Pharmacopoeia. Murine macrophages, RAW 264.7, and melanoma B16-F10 cell lines were cultured according to standard procedures. Cells were treated with either 5c, 6c or 30c ***Zincum metallicum*** and control cells with its respective vehicle (5c, 6c, or 30c Lactose). Macrophages activation by CD54 immunolabeling, intracellular reactive oxygen species (ROS) using DCFH-DA, and cytokines production using CBA were detected by flow cytometry. Phagocytic capacity was quantified by light microscopy. Features of melanoma cells were analyzed by colorimetric assays to determine melanin content and cell proliferation rate. All obtained data were submitted to normality test followed by statistical analysis.

Results: ***Zincum metallicum*** 6c shifted high ROS-producing macrophages to a low ROS-producing phenotype. Positive CD54 macrophages were increased by ***Zincum metallicum*** 5c. No changes in cytokine production and endocytic index were observed.

Conclusions: Differing responses and non-linearity were found on macrophages challenged with ***Zincum metallicum*** high dilutions. Melanoma cells were not affected by any treatment we tested. Customised assays using target cells can be useful to describe high dilutions effects. Other cell types and conditions should be investigated. *Homeopathy* (2017) ■, 1–11.

Keywords: Homeopathy; Highly diluted compounds; Macrophages; Melanoma

Introduction

The increase in understanding of cell structure and function has improved patients' chances of cure as scientists develop new therapies targeting specific molecules and cells. According to U.S. Food and Drug Administration (FDA), the drug development process must follow several steps, including basic research in association with clinical trials, directed to test drugs safety and efficacy.[1] Investigative toxicology is nowadays focused on defining better approaches to drug testing, including *in vitro* and surrogate models, molecular toxicology, 'omics' technologies, translational safety biomarkers, as well as preclinical imaging.[2] Cell-based models containing one or few cell lines are normally used in drug screening as a reliable source of safe evidence,[3] although it must be analyzed taking its limitations into consideration.[4]

It is long known that biological reaction rely not only on hydrogen, carbon, oxygen, nitrogen, and sulfur to properly run, but also an appropriate balance of trace elements are essential for life.[5] Zinc is one of the essential trace elements that plays important roles[6] in several proteins structure and functions such as metalloproteases,[7] DNA related proteins,[8] and oxidative stress.[9] Zinc transporters are

*Correspondence: Carolina Camargo de Oliveira, Laboratory of Inflammatory and Neoplasic Cells, Cell Biology Department of Biological Sciences, Universidade Federal do Paraná, Av Cel Francisco H dos Santos, s/n, CEP 81530-980, Curitiba, PR, Brazil.
E-mail: krokoli@ufpr.br
Received 7 July 2016; revised 9 February 2017; accepted 15 February 2017

normally found in cell membranes directing this element to inner cell compartments where it is required to maintain physiological functions. Any misbalance or dysregulation associated to these transporters can lead to loss of health and progress to several diseases.[10]

Homeopathy was originated late on the 18th century directed to healing process, and since then has been used by patients all around the world. The first basic research experiment was reported on the 19th century. Although over the past 3 decades the number of peer reviewed publications in this field has increased 3.5 fold,[11] researchers still need to determine how high dilutions work at cellular and molecular levels and must perform replications by independent groups.

Despite the fact that zinc has long been used in traditional and complementary medicine, few cases are published describing its effects when homeopathic preparations are used. Gibson, in 1967, described clinical features of *Zincum metallicum* including physiognomy, psychology, physiology, and pathology.[12] In a case study of diabetic peripheral neuropathy, a patient was subjected to different protocols and the best results were observed with *Zincum met* 6.[13] Badulici and colleagues have reported significant improvement in patients suffering from liver cirrhosis after *Zincum metallicum* 5c treatment, improving serum zinc levels, and favoring immunoglobulin G and M modulation.[14] Experimental models were also reported. *In vivo* treatment of mice bearing induced Ehrlich tumor ascites showed that *Zincum met* 200c increased animals life span.[15] More recent, induced parksonian rats presented amelioration on locomotor activity after *Zincum metallicum* 6c treatment, in addition to restored antioxidants defense.[16]

Normal and affected cells were already described by several groups to be sensitive to highly diluted compounds,[17] and our group has been working with cell-based assays for some time.[18−22] Thus, we sought to investigate common experimental end-points using cell-based models to determine the effects of *Zincum metallicum* in different potencies. Primary macrophages, Raw 264.7, and B16-F10 cell lines were used to test 5c, 6c, and 30c *Zincum metallicum*, along with its grinding mixture − Lactose − in the same potencies. Intrinsic properties of each cell type were observed and changes in these parameters were analyzed.

Material and methods

Testing solutions, cell culture and treatments

Zincum metallicum was prepared in lactose according to The Brazilian Homeopathic Pharmacopoeia, and kindly provided by Dr Carla Holandino Quaresma from Universidade Federal do Rio de Janeiro. Potencies of 5c, 6c, and 30c were achieved diluting original zinc solution in cell culture grade water (Sigma−Aldrich, St. Louis, MO, USA − #W3500). Dynamization was performed in 15 ml sterile polypropylene tubes (Corning, Corning, NY, USA) using DENISE automated machine (AUTIC, Campinas, SP, BR). Solutions were 100 times succussed between dilutions, yielding in a centesimal solution dilution. Final solutions were filtered sterilized by 0.22 μm syringe filter (Millipore, Billerica, MA, USA) and stored protected from light at room temperature. Lactose potencies were prepared the same way and used as controls.

RAW 264.7 murine macrophage cell line (ATCC, Manassas, VA, USA − #TIB-71) and primary macrophages were maintained in complete Dulbecco's modified Eagle's Medium − DMEM (Sigma−Aldrich, St. Louis, MO, USA − #D6429), containing 3.7 g/L sodium bicarbonate (Gibco, Waltham, MA, USA − #25080-094), supplemented with 10% heat inactivated fetal bovine serum (Gibco, Waltham, MA, USA − #12657029). B16-F10 murine melanoma cell line (BCRJ, Rio de Janeiro, RJ, BR − #0046) was maintained in complete DMEM, containing 1.5 g/L sodium bicarbonate, supplemented with 10% non inactivated fetal bovine serum. All media were supplemented with 1 U/ml penicillin, and 1 μg/ml streptomycin (Gibco, Waltham, MA, USA − #15140-148) and cells were maintained at 37°C in humidified atmosphere containing 5% CO_2. Cell lines were kept under 70% confluence for no more than 10 passages.

In general, cells were plated at optimal assays concentrations in cell culture appropriate flasks or plates (Sarstedt, Nümbrecht, DE). Six hours later, the cells received 20% v/v of fresh-succussed treatment. An 1% v/v booster dose was added every following 24 h, until completion of 48, 72, or 96 h of treatment for macrophages or melanoma cell lines.

Macrophages assays

Cell activation features by flow cytometry: RAW 264.7 cells previously stimulated or not with 200 ng/ml LPS (Sigma−Aldrich, St. Louis, MO, USA − #L4391) for 18 h were treated for 48 h and cells surface ICAM-1 (CD54) was detected by flow cytometry. Cells were scraped off, washed with PBS, and one million cells per group were incubated for 45 min with CD54-PE anti-mouse antibody (BD Biosciences, Franklin Lakes, NJ, EUA − #553253) at 4°C, and fixed in 2% paraformaldehyde.

Also, 48 h treated RAW 264.7 cells pre-treated or not with 200 ng/ml LPS were used to determine intracellular reactive oxygen species (ROS) production using flow cytometry.[23] One million cells per group were washed with 37°C PBS and incubated with 1 μM DCFH-DA probe solution (Sigma−Aldrich, St. Louis, MO, USA − #35845) for 30 min at 37°C, 5% CO_2 atmosphere. As a positive control, cells were incubated with 3 mM hydrogen peroxide (12,5 M H_2O_2 − Vetec, Duque de Caxias, RJ, BR − #194) for 5 min. Cells were scraped off and washed with PBS before flow cytometry reading.

Cytokine production by primary murine peritoneal macrophages was evaluated as previously described.[21] Macrophages were collected from untreated mice (sham group) of *in vivo* experiment previously approved by the ethics animal experiment committee of UFPR (number 694), reducing animal use in experimentation. Briefly,

Please cite this article in press as: Gonçalves JP, et al., Effect of *Zincum metallicum* on cell models I, Homeopathy (2017), http://dx.doi.org/10.1016/j.homp.2017.02.004

Download English Version:

https://daneshyari.com/en/article/5565268

Download Persian Version:

https://daneshyari.com/article/5565268

Daneshyari.com