The soundness of homeopathic fundamental research

Leoni Villano Bonamin·

Abstract

Fundamental research in homeopathy has much advanced in the past 20 years. From exploratory studies with animals and plants to the characterization of the systemic effects of homeopathic medicines and *in vitro* studies with isolated cell systems to assess changes in the mechanisms of cell adaptation and intracellular signaling facing variable homeopathic treatments. The amount of articles published over time enabled several systematic reviews. Recently, demonstration that homeopathic medicines might modify cell functions through epigenetic mechanisms (DNA methylation and demethylation) paved the road for a fully new field of research. In parallel, the discovery of nanoparticles and specific physical properties of homeopathic dilutions brought light to a previously poorly known field, as it was believed that homeopathic dilutions consist in nothing but water. Thus being, challenges for the future concern the demonstration, or not, of the interrelationship between both phenomena.

Keywords

Fundamental research; Homeopathy; Experimental models; Nanoparticles; Epigenetics

·Veterinary doctor, PhD; Universidade Paulista (UNIP), São Paulo, Brazil. ✉ leonibonamin@unip.br

The story begins by a question we have being hearing for decades: "Is homeopathy synonym of placebo?" This old controversy was elucidated in the past years, as the scientific literature included in database PubMed shows, especially as concerns meta-analyses of clinical trials [1-11]. Yet not only clinical trials provide scientific grounds to homeopathy. Along the past 10 years, considerable advance was made in fundamental research, most such studies having been performed in Brazil, Italy and India, seeking to elucidate the mechanisms of action of homeopathy.

Among recent systematic reviews [12-15] the ones assessing the reproducibility of studies conducted with dilutions above Avogadro's number stand out, including many different biochemical, immunological, botanical, cell biology and animal experimental models.

An analysis of studies performed in 2010 considered the (internal, independent or multicenter) reproducibility of studies [14]. A total of 107 studies were located, from which 53 exhibited comparable effects (35 internal, 8 multicenter and 10 independent repetitions); 8 studies exhibited consistent effects, however, not exactly the same as the ones in their predecessors; and 17 studies did not report any reproducible result.

A new survey was performed in 2015 of studies published from 1994 to 2015 [15]. A total of 126 experiments were located, 98 of which subjected to replication. Among the latter, 69 studies reported comparable effects, 20 no effects and 9 opposite effects. Statistical analysis led to reject the null hypothesis. About 82.9% of the studies exhibited internal reproducibility, 75% multicenter and 48.3% external or independent reproducibility.

Also plant models afforded relevant data on the reproducibility of results and on the pathophysiological mechanisms involved in the response to stressors following treatment with homeopathic drugs. A review from 2011 [16] which surveyed studies performed from 1920 to 2010 retrieved 34 articles fit for analysis according to the Manuscript Information Score (MIS). The articles were published from 1965 to 2010. A total of 37 experiments were described; 22 described data subjected to statistical treatment. Reproducible effects were found for decimal and centesimal dilutions, including potencies above Avogadro's number. Only one study with independent replication reported opposite results between the participating laboratories.

From 2000 onward, a considerable number of studies conducted with *in vivo* and *in vitro* experimental models were published, resulting in sufficient articles included in database PubMed for systematic review starting 2010. A systematic review performed by us in 2010 on animal experimentation [12] showed that the methods used until then were sufficiently adequate to obtain reliable data. Most such data exhibited convergence with the information in the homeopathic materia medica, i.e., the main tool used in clinical practice. The experimental models employed medicines prepared according to the isopathy and similitude (homeopathy) principles. In both cases it was possible to understand the complexity of the systemic actions of medicines, especially as concerns the modulation of the host-parasite relationship and the recovery of the body stability in the face of aggressive stimuli, which could be also corroborated through mathematical models.

The follow up of the aforementioned study was published in 2015. This new study reviewed articles on animal experimentation with homeopathy from 2010 to 2015 [13]. A total of 53 studies were located, relative to 12 different animal species; 29 studies used dilutions above Avogadro's number. Only 2 studies reported negative results, 1 with fish and 1 with bees; both employed commercial combinations of homeopathic medicines. The studies published after 2010 exhibited greater technical refinement, including association to results obtained also *in vitro* and 3 or more replications. A summary of the main findings of the reviews is described in Table 1.

Table 1. Summary of the main findings of 2 systematic reviews of animal models for homeopathic research published from 2010 to 2015 [12,13]

Parameters	**Articles published in 2010 [12]**
Total number of experiments	10 on isopathy 23 on similitude
Percentage of randomized samples	100%
Blind protocol	23 yes 10 no
Correlation between blind protocol and positive/negative outcomes	No (p= 0.6456, Fisher's test)
Convergence of experimental results and materia medica	87% for the studies on similitude
Parameters	**Articles published in 2015 [13]**
Total number of articles	53; 29 with dilutions above, and 10 with dilutions below Avogadro's number
Number of investigated species	12
Positive outcomes	100% for studies above Avogadro's number 80% for studies below Avogadro's number
Percentage of randomized samples	82%
Blind protocol	43%
Internal reproducibility	11%

In the past years, a patent trend to prioritize studies *in vitro* or with methods alternative to the use of animals developed, being encouraged by the main journals for complementary medicine, including *Homeopathy* [17]. The need to prioritize *in vitro* studies corroborating clinical results or obtained in animal models resulted in an interesting characteristic of the homeopathic phenomenon, already mentioned in previous reviews [13] but not taken into account until that point, to wit, translationality. This aspect allows for results obtained *in vitro* or in animal and plant models to generate information with immediate clinical applicability.

In 2017 we published 2 articles in journal *Cytokine* which clearly demonstrate this aspect. In the first article [18] we reported that macrophage-*Leishmania amazonensis* co-culture treated with *Antimonium crudum* 30cH *in vitro* exhibited significant reduction of lysosome activity, demonstrated through morphological analysis of the cells on fluorescence microscopy. We also found that treatment of infected cells

significantly reduced the peak of release of a chemokine crucial for monocyte recruitment in the inflammation site, to wit, MCP-1 (or CCL2) which only occurs in infected cells. However, we did not find any indication that treatment increased digestion of parasites. Considering the translational nature of the homeopathic phenomenon, these findings mean that in a hypothetical clinical situation, treatment of patients with *Antimonium crudum* 30cH might result in improvement of inflammatory lesions, but not in the elimination of infection.

Curiously, we had reached the very same conclusion in a previous, *in vivo* study, based on histopathological assessment [19]. Such result led us to ponder whether use of *Antimonium crudum* 30cH might be interesting, from the epidemiological point of view, to potentiate the parasiticide efficacy of the chemotherapy agents traditionally used for treatment of leishmaniasis. The reason is that by interrupting monocyte migration to the primary lesion, treatment might arrest the parasite cycle and proliferation in the definitive host, in this case the patient. The result might be greater parasite vulnerability to parasiticide agents and reduction of the duration of chemotherapy treatment, and thus of the its toxicity. It is superfluous to observe that reproducible, randomized, double-blind clinical trials are needed to validate this hypothesis. However, an *in vivo* study with a malaria model suggests this idea might be plausible [20].

Our second study [21] shows that treatment of mice with uropathogenic *E. coli*-induced experimental cystitis treated with *Cantharis vesicatoria* 6cH induced changes in the distribution of the various leukocyte subtypes along the urinary tract mucosa. The bladder mucosa exhibited predominance of B cells compared to all other cell subtypes, while the pelvic mucosa exhibited greater concentration of T lymphocytes and macrophages. High concentration of B lymphocytes in the bladder implies greater local IgA production which facilitates the control of infection in the lower urinary tract. This phenomenon would impair the propagation of infection to the kidney, i.e., so-called "ascending infection", which is usually attributed poor prognosis. Also in this case the experimental data awakened interest in the performance of randomized clinical trials to corroborate the clinical application of these findings.

The crux of the matter is that in none of those studies we found an 'antibiotic' effect, but facilitation of the host's adjustment to pathogens. Studies on parasitology conducted with laboratory animals treated with homeopathic and isopathic medicines corroborate this inference [22-27]. These data taken together with all other recent fundamental research studies reveal phenomenological particularities of homeopathic treatment that are not comparable to the phenomena observed in classic pharmacological studies, therefore, such particularities need to be taken into account in the design of clinical protocols. Among such particularities, non-linearity, coordinated systemic effects and probable epigenetic regulation are the most relevant, as shown by the series of studies conducted by the group chaired by Paolo Bellavite, from University of Verona, Italy [28-34].

Bellavite's group investigated 2 medicines, *Gelsemium sempervirens* and *Arnica montana*. The studies with *Gelsemium* found a non-linear anxiolytic-like effect in mice [29], i.e., without direct dilution-effect relationship. The behavioral changes observed were compatible with the ones described in the homeopathic materia medica. Two years later, these authors published an *in vitro* study conducted with SH-SY5Y cells in which treatment with *Gelsemium* in dilutions 2c to 30c modulated several genes involved in neuronal functions [31].

A clinical trial [32] exhibited considerable effectiveness of *Arnica montana* versus placebo, including improvement of post-traumatic pain, swelling and ecchymosis. In parallel, *in vitro* studies with human M2–polarized THP-1 macrophages through sensitization with PMA and interleukin (IL)-4 showed that treatment with *Arnica* modulated the expression of various genes involved in the regulation of chronic inflammation, such as a CXCL1, CXCL2, IL8 and BMP2, which encode vasoactive chemokines and cytokines [33]. In another experiment, dilutions 2c to 15c upregulated genes HSPG2, FBN2 and FN1, involved in the modulation of the extracellular matrix with participation in wound healing. The results also evidenced downregulation of some genes related to the aerobic metabolism, which suggests regulation of oxidative activity and consequently probably of *in vivo* tissue damage. In addition, *Arnica montana* 2c increased cell migration [34]. These findings corroborate the ones of previous *in vivo* studies, in which the action of *Arnica montana* 6c on vascular dynamics in acute inflammation proved to depend on individual variations [35].

Recently, the group chaired by Professor Anisur Khuda-Bukhsh, in India, showed, in cultures of various tumor cell lines, that the regulatory activity of several homeopathic medicines on gene expression occurred through epigenetic mechanisms, such as methylation/demethylation, triggering of pro-apoptotic mechanisms and regulation of telomerase activity [36,37].

In addition to the intracellular environment, also physical-chemical properties of the solvent used for preparation of highly diluted medicines are the focus of recent studies. Starting 2010, when presence of myriads of nanoparticles of variable nature suspended in homeopathic high dilutions was first reported [38], the idea quickly arose that the mechanism of action of homeopathic medicines might be related to nanopharmacology. This finding was repeatedly detected in the past years, particularly in experiments conducted in India [39-41].

In parallel, Demangeat, in France, identified nanobubbles in solutions subjected to agitation [42] which might also act as intracellular nanovectors.

Recently, studies conducted by Steven Cartwright [43,44] found that agitated solutions are associated with changes in the dipole activity of the water used as vehicle. This finding corroborates the hypothesis of electric resonance between medicine and intracellular water. Possibly nanoparticles also participate in this process.

However, it is still not known which of the aforementioned factors are truly determinant for modulation of cell functions to occur in such a refined manner. It is neither known how the information contained in medicines is 'decoded' by living systems at the systemic and epigenetic levels.

To summarize, the homeopathic phenomenon exhibits well-defined peculiar characteristics in which the rationale underlying classical pharmacology (dose-dependence) does not apply. Thus being, a new theoretical basis was suggested by Bastide and Lagache in the 1980s and 1990s, based on fundamental concepts of biosemiotics [45]. Application of the recent experimental findings to this conceptual basis represents a possible path for understanding how the similitude principle works in living beings in a highly specific manner [46]. Yet, this is a long path that we still need to tread.

References

1. Shang A, Huwiler-Müntener K, Nartey L, et al. Are the clinical effects of homoeopathy placebo effects? Comparative study of placebo-controlled trials of homoeopathy and allopathy. Lancet. 2005;366(9487):726-32.
2. Pakpoor J. Homeopathy is not an effective treatment for any health condition, report concludes. BMJ. 2015 350:h1478.
3. Frass M, Friehs H, Thallinger C, et al. Influence of adjunctive classical homeopathy on global health status and subjective wellbeing in cancer patients - A pragmatic randomized controlled trial. Complement Ther Med. 2016; 25:120-5.
4. Witt CM, Lüdtke R, Baur R, Willich SN. Homeopathic medical practice: long-term results of a cohort study with 3981 patients. BMC Public Health. 2005;5:115.
5. Mathie RT, Wassenhoven MV, Jacobs J, et al. Model validity and risk of bias in randomised placebo-controlled trials of individualised homeopathic treatment. Complement Ther Med. 2016;25:120-5.
6. Vithoulkas G. Serious mistakes in meta-analysis of homeopathic research. J Med Life. 2017;10(1):47-9.
7. Mathie RT, Clausen J. Veterinary homeopathy: systematic review of medical conditions studied by randomised trials controlled by other than placebo. BMC Vet Res. 2015;11:236.
8. Mathie RT, Van Wassenhoven M, Jacobs J, et al. Model validity of randomised placebo-controlled trials of individualised homeopathic treatment. Homeopathy. 2015;104(3):164-9.
9. Mathie RT, Frye J, Fisher P. Homeopathic Oscillococcinum® for preventing and treating influenza and influenza-like illness. Cochrane Database Syst Rev. 2015;1:CD001957.
10. Mathie RT, Clausen J. Veterinary homeopathy: meta-analysis of randomised placebo-controlled trials. Homeopathy. 2015;104(1):3-8.
11. Mathie RT, Lloyd SM, Legg LA, et al. Randomised placebo-controlled trials of individualised homeopathic treatment: systematic review and meta-analysis. Syst Rev. 2014;3:142.
12. Bonamin LV, Endler PC. Animal models for studying homeopathy and high dilutions: conceptual critical review. Homeopathy. 2010;99(1):37-50.
13. Bonamin LV, Cardoso TN, de Carvalho AC, Amaral JG. The use of animal models in homeopathic research--a review of 2010-2014 PubMed indexed papers. Homeopathy. 2015;104(4):283-91.
14. Endler P, Thieves K, Reich C, et al. Repetitions of fundamental research models for homeopathically prepared dilutions beyond 10(-23): a bibliometric study. Homeopathy. 2010;99(1):25-36.
15. Endler PC, Bellavite P, Bonamin L, Jäger T, Mazon S. Replications of fundamental research models in ultra-high dilutions 1994 and 2015--update on a bibliometric study. Homeopathy. 2015;104(4):234-45.
16. Jäger T, Scherr C, Shah D, et al. Use of homeopathic preparations in experimental studies with abiotically stressed plants. Homeopathy. 2011;100(4):275-87.

17. Chatfield K, Mathie RT, Bonamin LV, Oberbaum M, Fisher P. The publication in Homeopathy of studies involving animal experimentation. Homeopathy. 2016;105(3):211-6.
18. Santana FR, Dalboni LC, Nascimento KF, et al. High dilutions of antimony modulate cytokines production and macrophage - *Leishmania (L.) amazonensis* interaction *in vitro*. Cytokine. 2017;92: 33-47.
19. Rodrigues de Santana F, de Paula Coelho C, Cardoso TN, et al. Modulation of inflammation response to murine cutaneous Leishmaniasis by homeopathic medicines: *Antimonium crudum* 30cH. Homeopathy. 2014;103(4):264-74.
20. Rajan A, Bagai U, Chandel S. Effect of artesunate based combination therapy with homeopathic medicine china on liver and kidney of Plasmodium berghei infected mice. J Parasit Dis. 2013;37(1):62-7.
21. Coelho CP, Motta PD, Petrillo M, et al. Homeopathic medicine *Cantharis* modulates uropathogenic *E. coli* (UPEC)-induced cystitis in susceptible mice. Cytokine. 2017;92:103-9.
22. Ferraz FN, da Veiga FK, Aleixo DL, Spack Júnior M, de Araújo SM. Different treatment schemes and dynamizations of Trypanosoma cruzi biotherapies: what information do they transfer to the organism in infected mice? Homeopathy. 2016;105(4):327-37.
23. Sandri P, Aleixo DL, Sanchez Falkowski GJ, et al. Trypanosoma cruzi: biotherapy made from trypomastigote modulates the inflammatory response. Homeopathy. 2015;104(1):48-56.
24. Rodrigues de Santana F, Coelho Cde P, Cardoso TN, Laurenti MD, Perez Hurtado EC, Bonamin LV. Modulation of inflammation response to murine cutaneous Leishmaniosis by homeopathic medicines: thymulin 5cH. Homeopathy. 2014;103(4):275-84.
25. de Almeida LR, Campos MC, Herrera HM, Bonamin LV, da Fonseca AH. Effects of homeopathy in mice experimentally infected with Trypanosoma cruzi. Homeopathy. 2008;97(2):65-9.
26. Aleixo DL, Bonamin LV, Ferraz FN, Veiga FK, Araújo SM. Homeopathy in parasitic diseases. Int J High Dilution Res 2014; 13(46): 13-27.
27. Cajueiro AP, Goma EP, Dos Santos HA et al. Homeopathic medicines cause Th1 predominance and induce spleen and megakaryocytes changes in BALB/c mice infected with Leishmania infantum. Cytokine. 2017;95:97-101.
28. Bellavite P, Signorini A, Marzotto M, Moratti E, Bonafini C, Olioso D. Cell sensitivity, non-linearity and inverse effects. Homeopathy. 2015;104(2):139-60.
29. Bellavite P, Conforti A, Marzotto M, et al. Testing homeopathy in mouse emotional response models: pooled data analysis of two series of studies. Evid Based Complement Alternat Med. 2012;2012:954374.
30. Olioso D, Marzotto M, Moratti E, Brizzi M, Bellavite P. Effects of Gelsemium sempervirens L. on pathway-focused gene expression profiling in neuronal cells. J Ethnopharmacol. 2014;153(2):535-9.
31. Marzotto M, Olioso D, Brizzi M, Tononi P, Cristofoletti M, Bellavite P. Extreme sensitivity of gene expression in human SH-SY5Y neurocytes to ultra-low doses of Gelsemium sempervirens. BMC Complement Altern Med. 2014;14:104.

32. Iannitti T, Morales-Medina JC, Bellavite P, Rottigni V, Palmieri B. Effectiveness and Safety of Arnica montana in post-surgical setting, pain and inflammation. Am J Ther. 2016;23(1):e184-97.
33. Olioso D, Marzotto M, Bonafini C, Brizzi M, Bellavite P. Arnica montana effects on gene expression in a human macrophage cell line. Evaluation by quantitative real-time PCR. Homeopathy. 2016;105(2):131-47.
34. Marzotto M, Bonafini C, Olioso D, et al. Arnica montana stimulates extracellular matrix gene expression in a macrophage cell line differentiated to wound-healing phenotype. PLoS One. 2016;11(11):e0166340.
35. Kawakami AP, Sato C, Cardoso TN, Bonamin LV. Inflammatory process modulation by homeopathic Arnica montana 6CH: the role of individual variation. Evid Based Complement Alternat Med. 2011;2011:917541.
37. Mondal J, Das J, Shah R, Khuda-Bukhsh AR. A homeopathic nosode, Hepatitis C 30 demonstrates anticancer effect against liver cancer cells in vitro by modulating telomerase and topoisomerase II activities as also by promoting apoptosis via intrinsic mitochondrial pathway. J Integr Med. 2016;14(3):209-18.
37. Saha SK, Roy S, Khuda-Bukhsh AR. Ultra-highly diluted plant extracts of Hydrastis canadensis and Marsdenia condurango induce epigenetic modifications and alter gene expression profiles in HeLa cells in vitro. J Integr Med. 2015;13(6):400-11.
38. Chikramane PS, Suresh AK, Bellare JR, et al. Extreme homeopathic dilutions retain starting materials: a nanoparticulate perspective. Homeopathy. 2010; 99:231-42.
39. Rajendran ES. Homeopathy a material science! Nanoparticle characterization of *Aurum metallicum* 6C, 30C, 200C, 1000C, 10000C, 50000C and 100000C. Int J Current Res. 2017;9(4), 48923-7.
40. Upadyhay RP, Nayak C. Homeopathy emerging as nanomedicine. Int J High Dilution Res. 2011;10(37):299-310.
41. Bhattacharyya SS, Das J, Das S, et al. Rapid green synthesis of silver nanoparticles from silver nitrate by a homeopathic mother tincture *Phytolacca decandra*. Zhong Xi Yi Jie He Xue Bao. 2012;10(5): 546-54.
42. Demangeat JL. Gas nanobubbles and aqueous nanostructures: the crucial role of dynamization. Homeopathy. 2015; 104:101-15.
43. Cartwright SJ. Solvatochromic dyes detect the presence of homeopathic potencies. Homeopathy. 2016;105(1): 1-11.
44. Cartwright S.J. Interaction of homeopathic potencies with the water soluble solvatochromic dye bis-dimethylaminofuchsone. Part 1: pH studies. Homeopathy. 2017;106:37-46.
45. Waisse S, Bonamin LV. Explanatory models for homeopathy: from the vital force to the current paradigm. Homeopathy. 2016;105(3):280-285.
46. Bonamin LV. Descobrindo como a homeopatia funciona. Available at:: www.biodilutions.com