The soundness of homeopathic fundamental research

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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

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The story begins by a question we have been 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]

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<tr>
<td>Total number of experiments</td>
<td>10 on isopathy 23 on similitude</td>
<td>53; 29 with dilutions above, and 10 with dilutions below Avogadro’s number</td>
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<tr>
<td>Percentage of randomized samples</td>
<td>100%</td>
<td>82%</td>
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<tr>
<td>Blind protocol</td>
<td>23 yes 10 no</td>
<td>43%</td>
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<tr>
<td>Correlation between blind protocol and positive/negative outcomes</td>
<td>No (p= 0.6456, Fisher’s test)</td>
<td></td>
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<tr>
<td>Convergence of experimental results and materia medica</td>
<td>87% for the studies on similitude</td>
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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

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