Fundamental Research

Towards understanding molecular mechanisms of action of homoeopathic drugs: an overview

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Abstract

The homoeopathic mode of treatment often encourages use of drugs at such ultra-low doses and high dilutions that even the physical existence of a single molecule of the original drug substance becomes theoretically impossible. But homoeopathy has sustained for over two hundred years despite periodical challenges thrown by scientists and non-believers regarding its scientificity. There has been a spurt of research activities on homoeopathy in recent years, at clinical, physical, chemical, biological and medical levels with acceptable scientific norms and approach. While clinical effects of some homoeopathic drugs could be convincingly shown, one of the greatest objections to this science lies in its inability to explain the mechanism of action of the micro-doses based on scientific experimentations and proofs. Though many aspects of the mechanism of action still remain unclear, serious efforts have now been made to understand the molecular mechanism(s) of biological responses to the potentized form of homoeopathic drugs. In this communication, an overview of some interesting scientific works on homoeopathy has been presented with due emphasis on the state of information presently available on several aspects of the molecular mechanism of action of the potentized homoeopathic drugs. (Mol Cell Biochem 253: 339-345, 2003).

Keywords: homoeopathy, ultra-low doses, placebo effects, similia principles, mechanism of action, gene expression

Abbreviations: RCT-randomized control trials; hsp-heat-shock proteins; NMR-nuclear magnetic resonance; cAMP-cyclic adenosine mono phosphate; G-proteins - guanine nucleotide binding proteins

Introduction

In 1776, more than two hundred years ago, a German physician Samuel Hahnemann (1755-1843) noticed during experiments on himself that, after taking the malaria remedy quinine, he experienced symptoms similar to those of patients with malaria. Similar other tests (later termed 'provings' in English and Arzneimittelprufungen in German) were repeated on himself, his family and friends and the basic or 'similia' principle, i.e. 'similia similibus curentur' or 'like cures like', was apparently confirmed. The results of his large-scale provings led Hahnemann to conclude that, if a compound caused symptoms in healthy volunteers, it should then also serve as a remedy for patients who suffer from such symptoms.

In course of his experiments, Hahnemann noted with great interest that diluting and vigorously shaking his remedies (a process later termed as 'potentization') often rendered the remedy more potent in terms of clinical response. In homoeopathic potentization procedure, the mother tincture (e.g. original extract of the plant if the drug is derived from the plant) is generally diluted with 99 ml of rectified spirit (90% ethyl alcohol) and given 10 'succussions' or 'jerks' to produce the potency I C. Similarly, 1 ml

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of the drug solution at potency 1 C is again added with 99 ml of 90% ethanol and 10 jerks given to produce the potency 2 C and in this way by successive dilutions and succussions, further potencies like 30 C, 200 C and beyond are produced. Therefore at high dilutions, say beyond potency 12 C (i.e. beyond Avagadro's limit, i.e. 10-23) the solution is unlikely to contain even a single molecule of the original drug material (i.e. the mother tincture). Hahnemann was perhaps not aware of such Avagadro's limit, but he believed that 'vital force' of a substance was somehow released by the process of 'succussion' to the 'vehicle' which now behaved as the medicine. But how the medicinal properties of the drug could be transferred to and apparently retained by the 'vehicle' could not be scientifically explained though serious efforts were sporadically made in the positive direction to understand how water could display some sort of memory, and that biological activity could be displayed in the absence of the original molecule linked with this activity[1-4]. The two basic tenets of homoeopathy (like cures like and memory of water) have been challenged periodically by scientists and non-believers, but homeopathy has managed to survive even after more than two hundred years of its inception. Thus, this became a challenging scientific field for researchers to demonstrate the efficacy of this extremely diluted form of medicine in a scientific manner in controlled clinical trials and to explore its mode of action within the domain of science. In the following, an overview of some interesting research on homoeopathy will be provided and some salient points regarding limitations and scope of studies towards understanding molecular mechanisms of action of the ultra low doses of the homoeopathic drugs, particularly the biological responses to the homoeopathic drugs, will be briefly discussed.

Methodological difficulties in clinical research

There is no fixed medicine for any particular disease in homoeopathy, but there are particular medicines for particular sets of symptoms. The symptoms of the diseases are therefore all important rather than the disease itself in the selection of the specific drug. A particular medicine among a group of medicines is to be selected critically based on totality of symptoms. Thus, the first difficulty that is encountered in carrying out clinical trials is the lack of randomized control trials (RCTs) which are usually carried out double blind and termed as gold standard of clinical trial methodology[5]. Therefore, as there is not a single specific treatment for a single disease, the conventional form of clinical trials would demand to conduct as many clinical trials for as many diseases and as many remedies used in homoeopathy in order to show casual efficacy for all of them[6]. Thus, in order to find out whether a homoeopathic approach is comparable or superior to a standard treatment in general and for all sorts of patients, a randomized comparison study would be called for. The question to be answered by a double-blind randomized controlled trial with 'placebo' control (i.e. 'vehicle of the drug) is whether the homoeopathic remedies as such are superior to 'placebo' treatment[7-10]. But in the long last, it often becomes difficult to establish the casual efficacy of homoeopathic remedies over and against 'placebo'. Thus statistical analyses and meta-analyses of controlled clinical trials[10-12] were taken recourse to for acceptance of positive clinical effect of a drug over 'placebo' on human subjects with a given disease (i.e. with similar set of symptoms). Following strict guidelines for methodological and experimental criteria, a critical survey conducted on a total of 186 controlled trials in human led to the

conclusion that homoeopathic interventions had an effect over 'placebo'. However, it was also shown that there exists, to some extent, some minor psychological effects, benefits or even detrimental effects after 'placebo' administration[13]. Many other clinical evidences have also been forwarded to suggest that potentized homoeopathic medicines have positive ameliorating effects on human patients[9-14-15] and are definitely better responsive as compared to 'placebos'.

Some controlled experiments and results

To understand homoeopathy is essentially an inter-disciplinary problem, concerning physics, biology and medicine. Physics, for understanding the possible modes of action of succussions of solutions which do not contain even one molecule of the working/drug substance, at least theoretically. Biology, for an explanation of the extraordinary biological sensitivity, including a mechanism that works according to Hahnemann's basic simile, and potency rules. And medicine, to understand, besides medical efficacy, the main role in revealing what 'placebo' effects are all about and similar phenomena of 'mind-body' interactions. The decisive point of homoeopathy is the argument that homoeopathic remedies are not solutions but successions of the efficient substance (or imprints instead of mixtures in case of 'globuli')[16].

Physical concepts

Excellent working hypotheses have been advocated to explain how the specific organization of solvents is able to retain and maintain some properties of the initial substance[17-20]. A Clathrate model based on dielectric and differential scanning calorimetric measurements has been proposed[4,17] to explain how medicinal properties can be transferred to 'vehicle' and possible physico-chemical differences between homoeopathic dilutions and the corresponding solvent can be predicted. Some clathrates exist even if their core molecules are removed or exchanged for solvent molecules. Since clathrates may behave like crystals, they may replicate themselves during the homoeopathic dilution process in a similar way to crystal growth[4,17]. And the oscillation of the effectiveness of homoeopathic solution along the serial dilution process[3] might be similar to the oscillatory nature of crystal growth[21]. Matsumoto[22] advocated on the basis of the clathrate model that cell-surface proteins are activated by the hydration-shell structure of molecules in some cases, and the interaction between cell-surface proteins and the putative clathrate-like hydrate microcrystals formed during the homoeopathic dilution process is suggested as a primary molecular mechanism of biological responses to homoeopathic medicines.

Davydov[18], who investigated solitons very carefully, postulated a 'soliton excitation model' which suggested that the homoeopathic drugs acted like solitons which are responsible for 'high temperature-superconductivity' as well as for the well-known extraordinary sensitivity of biological systems.

On the other hand, various models based on physical properties of water and alcohol ('vehicle' of the homoeopathic drugs) have also been propagated to explain how the medicinal properties can be transferred and retained in the 'vehicle[19,20]. Further, structural differences between NMR spectra of homoeopathic potencies (say of sulphur) and their solvent ethanol could also be demonstrated by several workers[2,23]. Therefore, the question of transfer and retention of medicinal properties in the highly diluted homoeopathic medicines has largely been satisfactorily explained within the confines of physical sciences.

Biological models

However, how these microdoses emanate biological responses could not be properly understood for quite long. The initial research goal of most of the biological experiments was directed to demonstrate that ultra low doses of the homoeopathic drugs do have visible and quantifiable effects in various systems of animal models against suitable controls. Attempts were also made later to explain the mechanism of biological responses to homoeopathic drugs, by conducting both in vitro and in vivo experiments, of which a few are mentioned below.

In vitro experiments

The main studies aimed at demonstrating biological action have been conducted in toxicology and immunoallergology[24]. The term 'hormesis' was proposed by Southam and Ehrlich[25] to describe 'a stimulatory effect of sub-inhibitory concentrations of any toxic substance on an organism'. The literary meaning of this Greek word is 'excitation by an impulse'. Subsequently, Stebbing[26] used this term to describe the stimulation of growth by low levels of inhibitors. A team of researchers under the leadership of Roeland van Wijk and Fred A.C. Wiegant carried out extensive in vitro studies in the University of Utrecht, using cultured mammalian cells in homoeopathy research to primarily understand the underlying molecular mechanisms of hormesis.

According to these researchers [27], 'the stimulation of a disturbed self-recovery by the application of the similia principle is considered to be the essence of homoeopathy'. In their experiments, human fibroblasts were exposed to a threatening condition of chemicals (arsenite and cadmium) or a physical (increased temperature) nature and extensive analyses were carried out with regard to processes which might by considered as self-recovery, that is, cells' potential of temporal increased proliferation and additional development of resistance to the threatening condition. Further, specific analyses were also made of a number of molecular processes, particularly of a special class of protein molecules which have a function in the prevention of damage and assistance in recovery: the stress proteins or heat shock proteins. This group of workers demonstrated the production of new hsp 70 stress proteins which could be suggested as a representative process for damage and recovery. Further, they also studied the effect of microdose of the threatening condition (increased temperature, arsenite) and an increase of sensitivity followed by a decrease was found. Thus it was shown that small doses could bring about a clear improvement of the activation of the synthesis of hsp 70. This effect was specific for damaged and recovering cells as undamaged cells did not have such effect. Population of cells were disturbed by exposing them to different threatening conditions, after which it was investigated to what extent (sub-optimum) self-recovery could be activated in each population by the administration of low doses of either one of these threatening conditions. It was found that the application of these low doses at subliminal conditions to damaged cells early in their recovery enhanced the synthesis of hsp 70 to different extent[28,29]. Interestingly, altered levels of mRNA for hsp was found to be produced by the treatment of microdose used as per similia principle. Therefore, ultra low doses of the same toxic substances could evoke suitable recovery responses at the molecular and physiological levels.

Studies on crystal induced inflammation as well as insulin-receptor activation by oxyanions have been utilized to understand how the hydrate structure of certain types

of silica could activate or some anaesthetic agents could activate specific type(s) of cell surface proteins directly or indirectly, due to their coincidental complementary structures[22,30].

In vivo experiments

Many experiments maintaining suitable controls have been done mainly with an objective to demonstrate the efficacy of potentized form of homoeopathic drugs most commonly by using mammalian models like mice and rats, and occasionally other higher mammals like cattle or horse. Roberfroid et al.[31] studied the action of Hahnemanian potencies of 9C Phenobarbital on 2-Acetylamoinofluorene induced (using Phenobarbital as 'promoter') and found that 9C Phenobarbital effectively reduced the formation of hepatocarcinoma in rats after chronic feeding of the 2AAF and PB for several weeks. However, these authors did not use any other parameters or suggest any mechanism of action of how 9C PB could ameliorate hepatocarcinoma. Recently, Biswas and Khuda Bukhsh[32] demonstrated the efficacy of potentized homoeopathic drug, Chelidonium, in amelioration of p-DAB induced hepatocarcinoma of mice and explained the possible mechanism of action of the homoeopathic drug in rendering protection against p-DAB induced tumor formation and genotoxicity in mice. Further, the possible molecular mechanisms involved in the process of amelioration by microdoses of Chelidonium have also been explained[33] in the light of their observations with a number of cytogenetical and tumor-marker enzyme parameters.

Cazin et al.[34] conducted a pharmacological study of the retention (in blood) and mobilization of arsenic (through feces and urine) after intra-peritoneal administration of decimal and centesimal dilutions of arsenic in arsenic intoxicated rats. Subsequently, Khuda-Bukhsh and his collaborators[35-40] made an extensive investigation on the efficacy of oral administration of potentized Arsenic Alb (30 C and 200 C) in reducing arsenic-induced genotoxicity in mice by taking into consideration not only various cytogenetical protocols like chromosome aberrations (CA), mitotic index (MI), micronuclei testing (MNT), sperm head anomaly (SHA) etc., but also by other biochemical protocols like protein profiles, arsenic depositions in tissues, enzymatic studies (like lipid peroxidase, acid and alkaline phosphatase, glutathione, glutamate oxalo acetate transaminase and glutamate pyruvate transaminase activities), DNA and RNA contents, histopathological studies etc. and pointed out the application of these studies in combating human sufferings in arsenic contaminated rural areas of India and some other countries where arsenic contamination has assumed alarming proportions. Datta et al.[41] also obtained positive results with potentized Cadmium Sulph (30 and 200) showing its ability to reduce cadmium induced genotoxicity in mice. The 200th potency appeared to have marginally greater efficacy than the 30th potency of Cadmium Sulph in reducing cadmium-induced genotoxicity and the pre- and post-administration of the drug was found to have the maximum efficacy followed by that of only post-feeding of the drug.

Sukul[42] demonstrated increase in serotonin and dopamine metabolites in mouse hypothalamus and increase in firing rates in specific neurons following oral administration of a homoeopathic drug and opined that homoeopathic drugs act through autonomous nervous system.

Bentwitch[43] demonstrated that specific immune response to high dilutions to KLH could be transferred in some strain of mice.

Banik and Khuda-Bukhsh[44] reported positive modulations of cytogenetical and haematological effects by ultra-low doses of Ginseng in whole-body X-irradiated mice. Similar anti-clastogenic action of potentized Arnica Montana, Ruta Graveolens, Hypericum and X-Ray had also been reported earlier by Khuda-Bukhsh and his collaborators in mice[45-47]. Similarly, the genotoxic ill-effects of ultrasonic sound waves in mice could also be ameliorated by the oral administration of potentized Arnica Montana[48].

Aguejouf et al.[49] demonstrated theromoembolic complications to persist for several days after a single-dose administration of aspirin in Wistar rats subjected to experimental thrombosis induced by laser beams. Subsequently, this group [50] also demonstrated potent antithrombotic effect of acetyl salicyclic acid in similarly induced experimental thrombosis. After the first claim made on human basophil degranulation by very dilute anti-IgE by Benveniste's group[3] became controversial[51], stimulatory effects of high dilutions of histamine on activation of human basophils induced by anti-IgE have once again been demonstrated by a multi-centre European trial and other independently carried out works[52-56].

Mechanism of action

Although many papers on experimental research in homoeopathy have so far been published from well-established laboratories demonstrating positive effects/ modulations[31,34,43,48,50,52,54-57], the mechanism of action, particularly of the higher potencies (i.e. above potency C12 exceeding Avagadro's limit) that can bring forth spectacular changes (for removing disease symptoms) in living organisms (human patients or experimental animals) has not been properly dealt with by most authors. This may mainly be due to a major scientific bottleneck. One great difficulty that stands for criticism is the lack of understanding of what actually happens after the highly diluted ultra-low dose of the drug is administered on tongue. Tracing the movement of the 'molecular imprint' through receptors or nerve cells in the absence of the original 'drug molecule' or pinpointing the mechanism(s) and pathway(s) of action of the drug after it is administered on the tongue of a patient or an experimental animal is simply impracticable. The use of radio isotope (say, of Sulphur) in this regard can also be of little help as the ultra dilution will effectively throw out all radio isotopic original drug molecules. This makes it clearly much more challenging a task than studying the course of action of an orthodox medicine, say allopathic drugs. The mechanism and pathways of action of the macromolecules they contain can be traced inside the animal body. Therefore, it is no wonder that the mechanism and pathways of action of potentized homoeopathic medicines have mostly been, at best, speculated sometimes without any scientific support or sometimes only with circumstantial evidences. A few such hypotheses to explain biological or medicinal responses may be worth mentioning. Fisher [58] opined that the mechanism of action of the homoeopathic drugs could have some relationship with the Mithridates effect, but he did not elaborate on the mechanism involved in such effect. Davenas et al. [3] advocated a 'molecular imprint' hypothesis of the 'vehicle' of potentized homoeopathic drug which made positive changes in IgE modulations and exocytosis of mast cells. However, this paper later became controversial. Several researchers[26-29] tried to explain the mechanism of action as due to 'hormesis' effect based on their excellent scientific experimentations. In fact, these researchers (Wijk et al.) made valuable contributions to proteotoxicity, as they demonstrated

formation of abnormal protein molecules as a result of exposure of cells to toxic substances like cadmium or arsenite, an important event leading to disordered cellular physiological processes, and therefore forming the basis of loss of functionality, cellular damage and cell death. They have further shown that in case of cell damage, the protector protein synthesis is stepped up by exposure of cells to an application of a low dose of damaging condition (i.e. arsenite or cadmium). According to these authors, the replenishment of protector proteins starts with the activation of protector-protein gene promoters on DNA by binding of specific DNA-binding factors, called heat shock transcription factors (HSFs), on their appropriate DNA sites. The binding of a promoter constitutes a signal that triggers transfer of information from DNA into mRNA, eventually leading to new proctor proteins. An autoregulation loop was suggested to form the basis of the damage-induced recovery process. In fact, in a series of papers, Khuda-Bukhsh and his collaborators[35-40] also showed a wide variety of changes in protein, several enzymes (like peroxidases, acid and alkaline phosphatases, GPT, GOT etc.), DNA and RNA contents along with other cytogenetical changes after injection of a single dose of arsenic trioxide in mice. These changes were protected (favorably modulated) by the oral administration of a homoeopathic drug derived from ultra-dilution of arsenic trioxide. Therefore, the findings of Wijk and Weigant[27-29] also corroborated well with the hypothesis proposed by Khuda-Bukhsh[59]. This hypothesis advocated that one of the main mechanisms and pathways through which the potentized homoeopathic drugs act could be by regulation of expression of some specific and relevant genes and the circumstantial evidences on which the hypothesis is based have been adequately discussed[59].

However, it would only be fair to point out that the regulation of eukaryotic gene expression in eukaryotes is an extremely intricate process and is still not completely understood; however, established evidences suggest that the potential control levels include: (i) transcription; (ii) RNA processing (splicing or other types of processing/modification); (iii) mRNA transport; (iv) mRNA degradation and storage; (v) translation; (vi) post-translational modulation of protein activity. The expression of a given gene is likely to be regulated at one or several of these levels (see[60] for details). Thus the regulatory action of the homoeopathic drugs envisaged could be in one or many of these steps, including on regulation of transcription initiation by various specific transcription factors, as 'activator/effector' molecule or as 'co-activator' or 'silencer' molecule, or by regulation of RNA processing/translation by splicing or by negative translational control/translational frameshifting, or through various translocation signals, clonal selection etc.

Therefore, it is difficult to suggest at this moment the precise manner in which the homoeopathic drugs favorably influence the relevant genes to transcribe and translate the required proteins/enzymes to rectify the conditions (symptoms) that appear in the temporarily abnormal state of functioning of these genes. The failure to express the desired effect of homeopathic drugs in the presence of Actinomycin D, a transcription blocker, would further support the contention [38,48] that one way via which the potentized homoeopathic drug acted must be through active transcription. By undertaking further studies with suitable models directed towards understanding the molecular regulatory mechanism, either by blocking pathways selectively, or by selective induction of some of well-studied abnormal/diseased conditions, the role of

homoeopathic drugs diluted within and beyond Avagadro's limit, can prove rewarding.

Future scope of research

There is ample scope of doing further research on molecular chaperones like hsp (which incidentally are the direct products of some specific genes) needed for polypeptide folding and transport under both normal conditions as well as in cells subjected to other environmental stress. Similarly further studies on immunology and allergology can have important bearings on understanding the molecular mechanism of homoeopathy. Studies on activities of various signal transduction pathways after administration of potentized homoeopathic drugs in combating disease symptoms or in animal models subjected to abnormal/ induced conditions, could also be rewarding to understand if the homoeopathic drugs generate specifix signals to alleviate disease symptoms (say, by measuring secondary messenger activity of cAMP or the role of G-proteins).

Experiments may also be suitably designed to learn if these ultra-diluted drugs have specific ligand-binding ability (for sending signals inside specific cells through ion channels). Additionally, the protective/ ameliorating role of homoeopathic drugs can be further tested on modulations of activities of specific oncogenes or their gene products in artificially induced cancer in experimental animals against suitable controls. The activities of apoptotic genes or tumor-suppressor genes (e.g. p53) and their products, or some marker enzymes indicating onset of metastasis (e.g. the activity of Matrix Metallo Proteinase or MMPs) in artificially induced cancer mice/ rats can give important clues for understanding the molecular mechanism by which the homoeopathic drugs can influence and rectify faulty expressions of specific genes in the abnormal/diseased state. In-depth studies to examine if homoeopathic potencies act in some way to activate/repress activity of specific transcription factors can also be helpful in understanding the molecular mechanism. Further, to gain insight into the exact mechanism of action as to how the micro doses could delay the onset of cancer and how it could regress cancerous tumors to a considerable extent, electron microscopic studies (both SEM and TEM) may also prove rewarding. Incidentally, our recent studies through SEM and TEM in liver sections of mice treated chronically with p-DAB and Phenobarbital revealed striking changes in their sub-cellular organelles and cell membrane ultra-structure as compared to normal controls and a remarkable protection/recovery noted in these experimental mice fed homoeopathic drugs like Chelidonium-30 and Carcinosin-200, individually and conjointly, and particularly pronounced protective effects were observed in mice fed both these drugs intermittently (Khuda-Buksh et al., unpublished data). Thus, well thought out research programmes in diverse directions will, hopefully, yield sufficient understanding of the molecular mechanisms of action of the ultra-low doses of homoeopathic drugs, which eluded researchers for over two hundred years now.

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