

## Gelsemium sempervirens effects in vitro: A bridge between homeopathy and molecular biology?

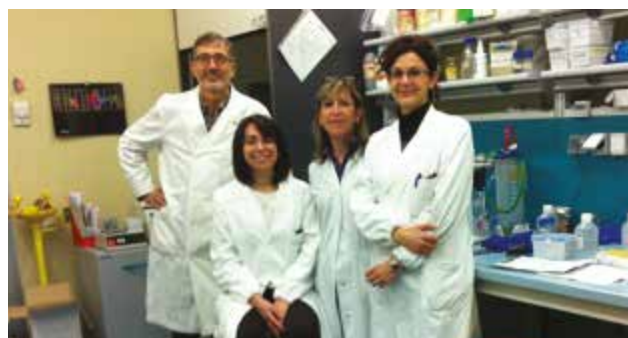
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### Introduction

Recent studies conducted in human/animal cells<sup>1-4</sup> have suggested that homeopathic remedies may have an effect on the gene regulation, initiating a cascade of gene actions to correct the disorder or disease at a cellular level. We have focused our attention on the possible gene modulation effect of *Gelsemium sempervirens*, a traditional medicinal plant employed in phytotherapy and homeopathy as a nervous system relaxant to treat various types of anxiety, pain, headache and other ailments. Previous investigations in our laboratory<sup>5</sup> have shown a significant anxiolytic-like activity of *Gelsemium s.* high dilutions in mice emotional models. To follow up on the above evidence, we further investigated the *Gelsemium s.* mechanism of action in *in-vitro* neuronal models in order to assess the effects of a wide range of drug dilutions on whole genome expression<sup>6-7</sup>. For this gene expression study we used sensitive microarray and real-time PCR techniques, able to survey the whole human transcriptome and specific genes of interest respectively. In our case the high-throughput microarray analysis assisted by bioinformatics could provide a strong clue as to the mechanism of action and the biological relevance of ultra-low doses, whereas the real-time PCR was useful in order to validate the microarray data and to further investigate a narrow group of genes, i.e. a panel of human neurotransmitter receptors and regulators, involved in neuronal excitatory signaling. To further investigate the possible mechanisms of gene regulation, we also analysed the possible effect of *Gelsemium s.* on the methylation status of a group of genes involved in mental disorders.

The chief innovation in our experimental design is that it employs a wide range of doses/dilutions: from low dilutions (2c or 3c), where the active substances can still be expected to exert their normal pharmaceutical action, to high dilutions (9c or 30c), where the most controversial principles of high dilution pharmacology come into play. We think that this type of investigation may be useful to start building a bridge between the more subjective point of view of symptomatology - basis of the homeopathic remedy choice - and a more accurate and objective one, consisting in the identification of specific molecular markers related with the disease and the prescribed remedy.



The Verona research group investigating complementary-integrative medicine on laboratory models. From left to right: Paolo Bellavite, Clara Bonafini, Marta Marzotto, Debora Oliosio.

### Experimental setup

Global changes in gene expression produced by exposure to high dilutions of *Gelsemium s.* extracts in human neuroblastoma cells were investigated by and real-time PCR techniques; the methylation analysis was conducted with a PCR Array.

Cells were incubated for 24h with the 6 dilutions of *Gelsemium s.*: 2c, 3c, 4c, 5c, 9c and 30c, produced by Boiron Laboratoires, according to the French Homeopathic Pharmacopoeia. Four replicate experiments were carried out under identical conditions.

### Gene expression experimental techniques

Depending on the type of cells and conditions in their immediate environment, cells will express different genes and at different levels. Microarray and RT-PCR analyses are two experimental techniques used to quantify this expression. Both start by isolating the RNA present in the cells, the concentrations of RNA being indicative of the level of activity of the corresponding genes.

**Microarray analysis** first translates these pieces of RNA into the corresponding DNA. These DNA sequences are then tested against gene probes which attach specifically to certain DNA sequences. Thus the presence of certain RNA sequences can be determined, providing information about gene expression levels in the cells.

**RT-PCR analysis** also starts by turning the RNA into DNA but then selectively amplifies a number of specific genes of interest. The genetic expression levels for these specific genes can then be measured. RT-PCR is able to investigate less genes but is more precise in its quantification, thus the two techniques are complementary.

#### Epigenetics in short

The expression of genes is modulated in different ways in the cell. One of the way cells turn genes on or off is through a process call methylation, which attaches methyl groups to specific locations along a gene thereby changing its expression. One of the current hypotheses in homeopathy is that homeopathic remedies might have an effect on the methylation states of genes, thereby turning them on or off. This would be most relevant in the treatment of chronic conditions where certain genes are being over or under-expressed.

## Results

Using microarray analysis, we observed that the diluted drug *Gelsemium s. 2c* significantly modulates the expression of 56 genes (49 were downregulated and 7 upregulated) involved in neuronal functions (G-protein coupled receptor signalling pathways, calcium homeostasis, inflammatory response and neuropeptide receptors). The expression of these genes also decreased significantly, although with small changes, after treatment with medium dilution (3c, 4c and 5c) and high dilutions of *Gelsemium s. 9c* and 30c.

In the study conducted using the real-time PCR technique (RT-PCR Array)<sup>7</sup> exposure of a human neurocyte cell-line to *Gelsemium s. 2c* dilution, containing the active principle gelsemine, induced a down-regulation of most genes of this array. In particular, the treated cells showed a statistically significant down-regulation of the prokineticin receptor 2, whose ligand is a neuropeptide involved in nociception (ability to sense pain), and in depression-like behavior. In the latter study, the 9c dilution was not active. The difference between the two gene-expression studies<sup>6-7</sup> is probably due to technical factors: while real-time PCR is the "gold standard" for gene expression analysis of specific genes or small groups of genes, microarray is a powerful screening method for the whole genome and in our conditions exhibited higher sensitivity, detecting extremely low dose effects.

In the epigenetic study the *Gelsemium s. 2c* treatment induced an increased methylation of the homeobox A1 gene (HOXA1), which has a role in neural development and autism spectrum disorders. The mean methylation frequency after treatment with *Gelsemium s. 2c* was 53% compared to 28% for the controls ( $p=0.008$ ). The increased level of methylation could indicate a reduced gene expression of this autism related gene.

## Conclusions

The results of this study, conducted with three different techniques, provided evidence that *Gelsemium s.* exerts a prevalently inhibitory effect on a series of genes across a wide dose-range. The results suggest the extreme sensitivity of human gene expression to regulation by ultra-low doses and high dilutions/dynamizations of a plant remedy and encourage further efforts in research on this field. Studies using sensitive genetic approaches may be particularly suitable for surveing of the effects of highly diluted natural compounds and for the identification of specific molecular markers related with the disease.

## Acknowledgements

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