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Proving the Effectiveness of Homeopathic Remedy Using a Cell-based System

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Abstract

Background: Homeopathy is a medical system based on the principle of "like cures like". It involves the use of natural substances that can cause a similar illness in a healthy person to stimulate the healing process. Homeopathic medicines use an ultra-diluted concentration of substances, which cannot be measured; therefore, their effectiveness is a matter of debate.

Methods: To prove the effectiveness of homeopathic remedies, mRNA and protein expression of cyclooxygenase-2 (COX-2) and pro-inflammatory cytokines were evaluated using RT-PCR and immunoblotting in primary cultured mouse chondrocytes and pre-osteoblastic MC3T3-e1 cells stimulated with a homeopathic dilution of *Rhus toxicodendron*.

Results: COX-2mRNA and protein were highly expressed in both chondrocytes and MC3T3-e1 cells stimulated with *Rhus toxicodendron*.

Conclusion: *Rhus toxicodendron* has been used as a homeopathic medicine to ameliorate arthritic pain and to modulate inflammatory conditions. During the inflammatory process, COX-2 is upregulated, which subsequently increases prostaglandin E2 production. Homeopathic dilution of *Rhus toxicodendron* increased COX-2 mRNA and protein expression, as confirmed by RT-PCR and immunoblotting. This study provides a unique approach for establishing the effectiveness of homeopathy; however, further studies are needed to examine the exact cellular signaling mechanisms.

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Homeopathy is a therapeutic method involving ultra-dilution of natural substances followed by succession. It was developed by Samuel Hahnemann, and is an alternative medicine system. This method is based on two main principles. The principle of 'like cures like', meaning that a substance that causes certain signs and symptoms in healthy individual scan also help to ameliorate those particular symptoms [1, 2]. The second principle is "memory of water", which is a concept by which the properties of an aqueous preparation of homeopathic remedies are held to depend on the history of the sample diluted beyond Avogadro's number [1, 3].

Numerous clinical trials and studies have been conducted to verify the effectiveness of homeopathic remedy by using experimental animal and cell-based model systems [4-6]. Recently, solvatochromic dyes have been used as molecular probes of serially diluted and agitated solutions to detect the potency of homeopathic remedies by using the visible spectrum of electronic spectroscopy [7]. Previously, we evaluated the effectiveness of homeopathic remedies using reverse transcription polymerase chain reaction (RT-PCR) and immunoblotting in primary cultured mouse chondrocytes and mouse pre-osteoblast [8,9]. In these studies, we used molecular biological methods to verify the effect of homeopathic remedy in cell-based model systems. These studies evaluated the gene expression, including the mRNA and protein expression of genes involved in the inflammatory response. The concept is that when amaterial reacts with the target cell, the intracellular signals, mainly in the nucleus, are stimulated in response. This response is a complex process involving various cellular membrane receptors and intracellular proteins. In response to extracellular stimuli, the nucleus can trigger rapid changes in the signals called gene expression (i.e., DNA → RNA (mRNA) → protein), despite the material being an ultra-diluted concentration of homeopathic remedy (Figure 1). Briefly, in this study, primary cultured mouse chondrocytes and mouse pre-osteoblastic MC3T3-e1 cells were treated with a homeopathic concentration of *Rhus toxicodendron* (*Rhus tox*), and the mRNA and protein expression of inflammatory-related cytokines, cyclooxygenase-2 (COX-2), and prostaglandin 2 (PGE2) were evaluated. Chondrocytes and MC3T3-e1 cell lines were grown for 24 h and stimulated with 2% ethanol or 4X, 30X, 30C, or 200C homeopathic dilutions of *Rhus tox* for 48 h. The expression of COX-2 was analyzed using RT-PCR, qRT-PCR and immune blot.

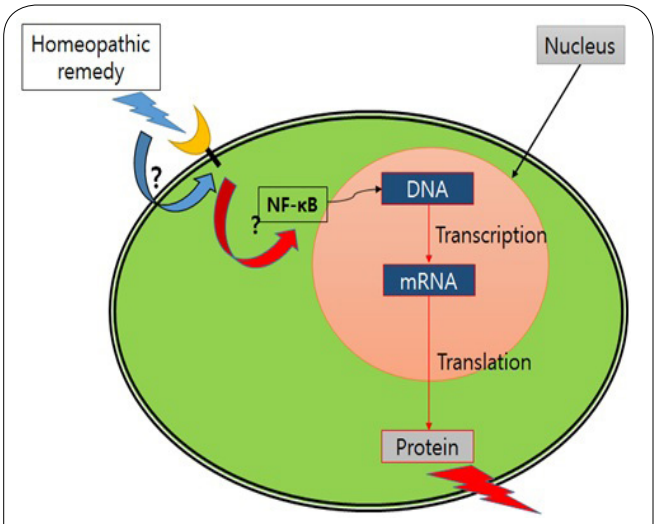

Figure 1. Possible signaling pathway of homeopathic remedy-stimulated gene expression in cultured cell lines. The extracellular stimuli stimulate the nucleus to trigger changes in gene expression (i.e., DNA → RNA (mRNA) → protein), despite the stimuli being an ultra-diluted concentration of homeopathic remedy that contains no detectable amount.

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The results showed that stimulation with Rhus tox increased COX-2 mRNA and protein expression in both cell lines, particularly with 30X *Rhus tox*, but 4X, 30C and 200C diluted *Rhus tox* did not elevate *COX-2* mRNA expression compared with the 30X *Rhus tox* stimulation, although the expression of *COX-2* protein was slightly increased in stimulated with 30C *Rhus tox* in MC3T3-e1 cell lines[8, 9]. In addition, treatment with 30X *Rhus tox* increased PGE2 release compared with other homeopathic dilutions. Collectively, these results show that stimulation of cells with *Rhus tox* induces the up-regulation of *COX-2* mRNA and protein, there by enhancing PGE2 production.

Rhus tox has been widely used as a traditional homeopathic remedy for the treatment of inflammatory conditions, including skin eruption, back pains and stiffness, irritability, restlessness, rheumatoid arthritis, and joint pain[10-12]. Several clinical trials have been conducted to demonstrate the anti-inflammatory and immunomodulatory activity of homeopathic dilutions of *Rhus tox* [4, 12, 13]. In an experimental animal model system, both the cellular and humoral immunity of *Rhus tox*-treated mouse were stimulated [14]. In addition, previous studies demonstrated the anti-arthritic activity of *Rhus tox* in its crude form and different homeopathic dilutions of Rhus toxin an adjuvant-induced arthritis model [12].

Inflammation is a protective response of the immune system, and it involves various pro-inflammatory cytokines such as tumor necrosis factor (TNF)-α and interleukin-1β (IL-1β), which subsequently trigger the release of other cytokines such as transcription factor nuclear factor (NF-κB)[15]. NF-κB regulated the expression of TNF-α, IL-1β, IL-6, CXCL8, and CXCL10, which induced the upregulation of COX-2, metalloproteinase, and phospholipase A2[16, 17]. It has also been reported that the anti-inflammatory action of non-steroidal anti-inflammatory drugs (NSAIDS) inhibits the activity of COX, thereby diminishing the synthesis of pro-inflammatory prostaglandins[17, 18]. COX has two distinguished isoforms, COX-1 and COX-2[18]. COX-1 is constitutively expressed in most tissues[18], whereas COX-2 is stimulated by inflammatory signals; hence, it is mainly involved in inflammation. COX-2 is primarily responsible for the synthesis of the prostanoids (prostaglandins and thromboxanes) involved in pathological processes [18-20]. It promotes the release of pro-inflammatory mediator PGE2, while COX-2 inhibitors suppress PGE2 production [21] . Our study was designed to verify the effectiveness of homeopathic dilution of *Rhus tox* by comparing with the results of previous studies. First, we tried to validate the doctrine of homeopathy: 'like cures like'. If a homeopathic remedy of *Rhus tox* reduces the inflammatory conditions, the remedy should induce inflammatory signals in normal cell lines. Secondly, the experimental approach focused on the mRNA expression of pro-inflammatory cytokines and COX-2 in cells treated with *Rhus tox*. Although this is not the first study to evaluate the immunomodulation effects of homeopathic remedies, it has important in homeopathic remedy application [22]. Previous findings showed that COX-2 was dramatically upregulated during inflammation in patients with rheumatoid arthritis, and an inhibitor of COX-2 exerted anti-inflammatory effects [23]. Our results clearly showed that *Rhus tox* increased *COX-2* mRNA and protein expression in chondrocytes and pre-osteoblasts. In particular, the concentration of 30X Rhus tox exhibited potent activity and decreased collagen type II expression[8, 9]. Further, 30X *Rhus tox* increased PGE2 productions. Taken together, these results suggest that the homeopathic dilution of *Rhus tox* has a direct effect on the inflammatory responses by modulating *COX-2* mRNA expression and PGE2 production in chondrocytes. These findings support the use of *Rhus tox* as a homeopathic remedy owing to its immunomodulatory activity, and it provides the scientific basis for homeopathy applications.

Competing Interests

The author declares that he has no competing interests.

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