Studies

# Immunomodulatory Effects of a Homeopathic Agent

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## Abstract

The in vitro and in vivo immunostimulatory effects of a homeopathic medication were investigated. Peripheral Blood Mononuclear Cells (PBMC) were isolated from normal controls and patients with either Chronic Fatigue Syndrome (CFS) or Acquired Immunodeficiency Syndrome (AIDS). They were then incubated for 24 H in the presence of a 1:10 dilution of the homeopathic mixture or 5% ethanol placebo. The homeopathic preparation significantly increased Natural Killer (NK) cell activity versus K562 cells in a standard 4 h 51CR release assay. This is in marked contrast to placebo for PBMC from normal controls ($p<.05$) or patients with CFS ($p<.01$) or AIDS ($p<.01$).

Either 5% ethanol placebo or the homeopathic medication was administered to groups of adolescents CD-1 mice at a dose of 0.35 cc daily for 28 days. None of the mice manifested any evidence of gross or microscopic toxicity (brains, kidneys, livers, pancreases, or hearts). Splenic NK function versus YAC-1 targets was significantly greater in mice treated with homeopathic agent (mean 103 +/- 10.9 lytic units [LU]; $p<.05$) compared to placebo (mean 81 +/- 7.4 LU). Groups of mice were treated with 21-, 14-, 7-, or 0-day courses of the homeopathic tincture or placebo. They were then challenged with 1 x 104 plaque-forming units (PFU) of a diabetogenic strain of coxsackie virus B4 (E2). Treatment was continued for an additional three days; then the mice were sacrificed. Titers of virus in the pancreas were significantly reduced in the homeopathic group that was treated for 21 days prior to viral challenge (mean [log 10] 3.14 +/- 0.79pfu/mg; $p<.05$) compared to placebo (4.29+/-0.90pfu/mg). The homeopathic mixture did not exhibit any antiviral effect in vitro. Thus, a homeopathic medication increased NK function both in vitro and in vivo and was non-toxic to mice. In vivo antiviral activity was demonstrated, presumably through immune enhancement.

## Introduction

Chronic Fatigue Syndrome (CFS) is a disorder of unknown etiology, characterized by persistent constitutional symptoms, severe fatigue, and cognitive defects.[1] The diagnosis is currently based upon clinical criteria established by the Center for Disease Control. Several hypotheses have been proposed to explain the cause of the disease, including psychiatric dysfunction,[2] hypothalamic-pituitary-adrenal glade disturbance,[3] immune dysregulation,[4] and/or viral infection.[5] Effective treatment has not been established.

Acquired Immunodeficiency Syndrome (AIDS) is the last stage of a disease caused by chronic infection with the Human Immunodeficiency Virus (HIV). The majority of affected patients develop progressive immune-system deficiency

over several years, eventually dying from one or more cancers or opportunistic infections.

Patients with CFS often have diminished NK cell function. Some studies have demonstrated a beneficial clinical effect of immune modulators.[6,7] HIV-infected individuals suffer from defective NK function, which progressively deteriorates with disease progression.[8] A clearly beneficial clinical effect with immune enhancers has not yet been demonstrated in these patients, although therapy with Interleukin-2 (IL-2) was beneficial in one study.[9] Thus, CFS and HIV are characterized by diminished NK function, a role for viruses is either proven (HIV) or postulated (CFS), and there is no cure. Therefore, it is not surprising that many patients resort to treatment modalities, including homeopathic preparations, that are outside the mainstream of Western medicine.

Key Terms

PBMC = Peripheral Blood Mononuclear Cells
AIDS = Acquired Immunodeficiency Syndrome
NK = Natural Killer
CFS = Chronic Fatigue Syndrome
HIV = Human Immunodeficiency Virus
IL = Interleukin
CVB =coxsackie B virus
LU = lytic units
PFU = plaque-forming unit
MK = monkey kidney
NS = not significant

Homeopathic remedies have been used since the late 18th century for a variety of ailments.[10] These medicines are made from plant, animal, or mineral preparations that have been diluted in a liquid base to micromolar concentrations. The components are typically toxic in larger concentrations. A general theory of the mechanism of action, although still unproven, involves a strengthening of specific host functions in response to noxious stimuli.[11] The efficacy of homeopathic formulations has never been definitively proven scientifically, although two meta-

analysis have both reported positive results in over 65% of clinical trials.[12] However, the majority of these trials were either poorly controlled or involved a small number of patients.

Some homeopathic preparations have postulated immune-enhancing effects, including the preparation used in the current study. This medication contains 5% alcohol and micromolar concentrations of Cactus grandiflorus, Aloe socotrina, Abies nigra, Arnica, Lachesis, calcium carbonate, and Licopodium. Anecdotal reports have suggested an improved survival and quality of life in some HIV-infected patients. In the current study, the homeopathic preparation was found to:

- Significantly stimulate the in vitro NK activity of PBMC from normal controls and patients with CFS or AIDS;
- Exert no toxicity when administered in high doses to mice;
- Significantly increase splenic NK function in mice; and
- Diminish infection in the pancreas of mice challenged with a diabetogenic strain of CVB4 (E2).

## Materials and Methods

### Medication preparation

Extracts of Cactus grandiflora, Aloe socotrina, Abies nigra, Arnica, Lachesis, and Licopodium were combined with calcium carbonate. The medicine was manufactured in accordance with the methods and specifications of the Homeopathic Pharmacopoeia of the United States. Specifically, each extract was serially diluted in 5% ethanol and sterile water to a final concentration of 1:106 and combined with the other components. The final mixture was potentiated by repeated vortexing. Spectrophotometriacal analysis of the final mixture revealed only ethanol and water. This is in accordance with the standard homeopathic practice of diluting the initial components to the point at which they cannot be detected by standard means.

### In vitro studies

#### Effect of the homeopathic medication on NK function

Preparation of PBMC: Heparinized blood was collected between 12:00 and 2:00 p.m. from 20 normal individuals picked from the staff at the University of California, Irvine Medical Center, and 20 sex-and age-matched patients with CFS (as defined by 1988 CDC criteria) or AIDS (CD4 count <200). Patients with CFS or AIDS were allowed to take prescription or over-the-counter medications. However, use of agents with known or suspected immunomodulating effect, such as corticosteroids, colony-stimulating factors, interleukin-2, interferons, or cancer

chemotherapy, was not permitted. Standard Ficoll-Hypaque density gradient centrifugation was used. 13 PBMC were suspended in complete RPMI media with 10% heat-inactivated fetal bovine serum (FBS). They were tested immediately.

NK function assay: A standard cytotoxicity assay assessed NK cell activity.[14] K562 cells, maintained in complete RPMI with 10% FBS (heat inactivated), were used at target cells. They were labeled with 20 uC51Cr (ICN, Costa Mesa, CA) for 1 h at 37 degrees C (5% CO2) and washed four times with medium. In addition, cell suspensions were pipeted at a concentration of 5 x 103 cells/well to 96-well U-bottom microtiter plates. Effector cells (PBMC) were added in triplicate to the wells at an effector:target ratio of 40:1, 10:1, and 5:1. This was followed by the homeopathic mixture at a total dilution of 1:10, or with ethanol, a final concentration of 0.5%. The effector cells had been preincubated with either additive for 24 h in some runs. Control wells without effectors contained target cells and either the homeopathic preparation or ethanol alone. This was to determine the spontaneous lysis, or 3% Triton X-100, for evaluation of total lysis.

After incubating for 4 h 15 37 degrees (5% CO2), the plates were centrifuged for 10 min at 1,500 x g, with 100 ul of the supernatant was removed and mixed with 2.5 ml of scintillation cocktail (Fisher Scientific, Tustin, CA). Liquid scintillation counting (Beckman LS-100) and cytotoxic activity determined radioactivity, calculated in terms of lytic units (LU) by a software program provided by H. Pross.[15] One LU is defined as the number of effector cells required to achieve 20% specific lysis of 5 x 103 targets. LU were calculated per 107 effector cells.

In vitro antiviral activity and toxicity: Virus activity was assessed in Monkey Kidney (MK) cell monolayers. It was evaluated in the presence of 5% ethanol or Liebovitz's L15 diluent (controls) or serial tenfold dilutions of the homeopathic medication with coxsackie virus B4 (CVB4) strain E2, at an inoculum of 1 plaque-forming unit (PFU) per cell. Cellular toxicity was determined by recording morphology and monolayer formation of MK cells grown in the presence of the undiluted homeopathic medication (final concentration of 20%).

## In vivo testing

Animals: Four-week old male CD-1 mice were obtained from Charles River Farms (Wilmington, MA).

Virus: CVB4 strain E2 was propagated and maintained as previously described. [16]

## Experimental methods

Toxicity study: Either placebo (5% ethanol) or the homeopathic mixture was administered to groups of 10 adolescent, male CD-1 mice. The animals were injected subcutaneously with a 0.35 cc of placebo or the active preparation on a daily basis for 28 consecutive days. The animals were observed daily for evidence of gross toxicity (ataxia, lethargy, respiratory distress). After 28 days, sections of harvested brains, kidneys, livers, pancreases, and hearts were fixed in 10% formalin,

embedded in paraffin, cut by microtome, mounted on slides, strained with hematoxylin and eosin, and examined under a light microscope for histopathologic changes.

In vivo effect of the homeopathic medication on stimulation of splenic NK function: CD-1 mice were injected daily for 28 consecutive days, by subcutaneous administration of either 0.35 cc of the homeopathic agent or 5% ethanol (placebo). Spleens were removed aseptically, pressed through stainless steel mesh grids, and suspended in complete RPMI medium. For 3 minutes 0.83% ammonium chloride was added to lyse erythrocytes. Mononuclear cells were separated from other cell populations by Ficoll-Hypaque centrifugation, suspended in complete RPMI medium, and used immediately as effector cells. NK-sensitive YAC-1 cells (American Type Culture Collection) were maintained in complete RPMI media and used as target cells. They were labeled with 50 uC 51Cr for 1.5 h at 370 (5% CO2), washed four times with media, and seeded onto 96-well microtiter plates at a concentration of 1 x 104 cells/well. Splenic effector cells were added in triplicate to the wells at effector: target ratio of 40:1, 20:1, 10:1, and 5:1. Radioactivity in 100 ul of supernatant was measured as above LU calculated.

In vivo antiviral effect: Either 0.35 ccl placebo (5% ethanol) or the homeopathic mixture was administered subcutaneously to mice on a daily basis for either 21, 14, or 7 consecutive days prior to challenge with virus, or starting on the day of virus inoculation. The animals were challenged intraperitoneally with 1 x 104 PFU CVB4 strain E2, and the treatment continued. Samples of pancreas were harvested 3 days after virus inoculation. Plaque assay determined the virus titer in the homogenized tissue. Control mice received the homeopathic medication only, without viral challenge for evaluation of toxicity. The remaining mice received 5% ethanol alone, and served as uninfected controls.

## Results

In vitro NK assay: The mean NK function was 103.7+/-22.1 LU for 20 normal controls. NK function was significantly decreased both for 20 patients with CFS (47.3+/-16.2;$p<.01$) and the 20 patients with AIDS (8.0+/-3.8;$p<.001$). A significant enhancement of NK function for all three groups was observed when PBMC—preincubated for 24 h with the homeopathic medication—were used as effectors compared to effectors without any additive (Table. 1). The effect was greatest for patients with CFS or AIDS ($p<.01$ for both). The addition of ethanol or the homeopathic medication without preincubation did not enhance NK function in any of the groups.

In vitro antiviral effect and toxicity: Replication of CVB4 strain E2 was not inhibited at any concentration of the homeopathic preparation. No MK cell toxicity (rounding of cells and/or monolayer disruption) was observed after incubation was undiluted homeopathic doses.

In vivo toxicity: No evidence of gross or microscopic toxicity was observed for any of the tissues examined.

In vivo stimulation of splenic NK function: Splenic NK function versus YAC-1 targets was significantly greater in mice treated with the homeopathic

medication for 28 days (mean 103+/-10.9LU;p<.05) compared to placebo (mean 81+/-7.4LU).

In vivo antiviral effect: Titers of virus in the pancreas were significantly reduced in the homeopathic group treated for 21 days prior to viral challenge (mean [log10] 3.14+/-0.79 pfu/mg; p<.05), compared to infected animals treated with a similar course of placebo (4.29 +/-0.90pfu/mg; Table 1). Treatment with the homeopathic mixture for 14 days or less prior to viral challenge did not result in a reduction in virus titer in the pancreas when compared to a similar course of placebo-treated mice.

Table 1

In vivo effect of a homeopathic preparation on viral titer in the pancreas of CD-1 mice 3 days after inoculation with CVB4, strain E2.

| Treatment | Medication | Placebo | |
|---|---|---|---|
| Start day of therapy | | | P |
| 0 | 4.36 =/-1.01 | 4.17 =/-0.96 | NS |
| -7 | 4.11 =/-0.85 | 4.33 +/-1.05 | NS |
| -14 | 3.95 +/-0.99 | 4.21 +/-0.79 | NS |
| -21 | 3.14 +/-0.79 * | 4.29 +/-0.90 | <.05 |

Results represent means for 10 mice/group. Animals were treated with 0.35 cc of placebo or the homeopathic medication subcutaneously from the start through the Sacrifice day.

* Titers <2 were assigned a value of 1 for purposes of determining the mean. NS=Not significant

## Discussion

The results of multiple anecdotal reports and uncontrolled or poorly controlled studies have suggested that homeopathic products may be beneficial for a variety of clinical conditions. These include diarrhea, allergic rhinitis, and influenza.[17, 18] The best evidence for efficacy is for asthma, for which three placebo-controlled trials using homeopathic preparations of appropriate allergens shows benefit. [19, 20]

Homeopathic products contain substances that have been diluted to such an extent that they are no longer detectable by mass spectrometry. The mechanism of action of these products has not been established. However, this fact should not discourage further investigation of homeopathy, since many other established therapies—such as allergy immunotherapy—act by unknown processes. Instead, homeopathy should be studies in randomized, placebo-controlled studies using established endpoints. Positive studies should be confirmed.

The results of the current study suggest that a homeopathic preparation made from a mixture of herbal, animal, and mineral products enhances NK function both in vitro and in mice. The positive results obtained in the in vitro study are particularly noteworthy, as evidence of in vitro activity of homeopathic products has not previously been established. In vitro investigations should assist in screening promising products prior to adequate clinical trials. The results also suggest that modifications of well-established in vitro assays may be required. This is possibly due to mechanisms of action for homeopathic products that are different from allopathic medications. For example, in the present study, a standard 4 h 51Cr-release NK function assay did not demonstrate efficacy of the homeopathic product.

Preincubation with the effector cells was required. The reason for this finding is unknown, and merits further investigation.

The homeopathic preparation was effective in stimulating in vitro NK function in patients with CFS and AIDS, disorders characterized by defective cellular immunity. Thus, trials to establish clinical efficacy in immune-based diseases is merited. Elaboration of the mechanism of NK stimulation is also warranted. Finally, the current investigation demonstrated that NK stimulation could be accomplished in vivo (using mice) and that this immune enhancement resulted in protection against an NK-sensitive virus, CVB4. However, potential use as an antiviral agent is hampered by the fact that a significant period of pre-treatment was required (21 days). Thus the homeopathic medication used in the current study may be more useful as a prophylactic agent rather than for treatment of acute viral infection. Notable exceptions would be persistent, NK-sensitive viruses such as hepatitis B and HIV.

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