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ORIGINAL PAPER

Therapeutic potential of HIV nosode 30c as evaluated in A549 lung cancer cells

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Objectives: **To examine if HIV nosode in 30c dilution (HIV 30c) has therapeutic potential against lung cancer cells (A549) as compared to WRL-68 normal cells and to elucidate its possible molecular mechanism of action on DNA replication and apoptosis.**
Methods: **Effects of HIV 30c were thoroughly tested for its possible anticancer potential on A549 cells (lung cancer); WRL-68 normal liver cells served as control. Three doses, one at LD50 and two below LD-50, were used. Proliferation, migration and senescence assays were made and generation of reactive oxygen species (ROS) studied by routine techniques. The ability of HIV 30c to induce apoptosis in A549 cells and its possible signalling pathway were determined using immunoblots of relevant signal proteins and confocal microscopy, including studies on telomerase reverse transcriptase (TERT) and topoisomerase II (Top II) activities, intimately associated with cell division and DNA replication.**
Results: **HIV 30c prevented cancer cell proliferation and migration, induced pre-mature senescence, enhanced pro-apoptotic signal proteins like p53, bax, cytochrome c, caspase-3 and inhibited anti-apoptotic signal proteins Bcl2, TERT and Top II, changed mitochondrial membrane potential and caused externalization of phosphatidyl serine. Thus, it induced apoptosis as also evidenced from increase in cells with distorted membrane morphology, nuclear condensation, DNA fragmentation, and ROS, typical of apoptosis in progress.**
Conclusion: **HIV 30c nosode has therapeutic potential for inducing cytotoxic effects on A549 cells as manifested by changes in nuclear condensation, DNA fragmentation, ROS generation and MMP, and for its inhibitory action on cell proliferation, cell migration, expression of telomerase reverse transcriptase and Top II genes, and increasing expression of pro-apoptotic genes.** *Homeopathy* (2017) ■, 1–11.

Keywords: HIV nosode; Anticancer potential; A549 cells; Telomerase reverse transcriptase; Topoisomerase-II

Introduction

Infection by human immunodeficiency virus (HIV) initially produces a brief period of influenza-like illness, but then the virus usually replicates a single stranded DNA by reverse transcriptase activity and gets incorporated into the host genome without producing any notable symptom for a variable period of time.[1] The gene product from the host genome then begins to cause a progressive loss of CD4+ T-cells, leading to almost total dysregulation of the immune system, and the victim now becomes extremely susceptible to common infections, like tuberculosis, and other opportunistic infections and tumours. The late symptoms of acquired immune deficiency syndrome (AIDS) often get complicated by a particular infection of the lung known as "pneumocystis pneumonia", associated with severe weight loss, skin lesions

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caused by Kaposi's sarcoma, or other AIDS-related conditions.[2] Therefore the lung becomes one of the final targets of this virus at later terminal stages of the disease.

Since the advent of AIDS disease in the early 1980s doubt about its viral origin persisted until the actual virus was detected[3] and its viral origin confirmed.[4] But in the first two decades, thousands of people died as no drug could resist the virus from replicating and attacking the immune system through its gene product.

Frantic searches were made initially for drugs that could possibly cure the disease to save the impending global catastrophe, but these proved unsuccessful. Attempts were then made to treat the disease using a defensive and preventive approach, by trying to stop replication of the virus to stall rapid spread of the disease. As reverse transcriptase activity was extremely important for virus replication and entry into the host genome, drugs like reverse transcriptase inhibitors, or protease inhibitors that could interrupt the virus from replicating, showed some response in limiting spread of the virus in the body, but unfortunately their severe side-effects restricted their use. The search was extended to other modes of treatment like complementary and alternative medicines (CAM). In the US, approximately 60% of people with HIV prefer to use various forms of CAM[5] even though the effectiveness of most of these therapies has not yet been firmly established.[6] Some homeopathic remedies have also been claimed to show ameliorative antiviral effects in the treatment and managements of AIDS in humans[7–9] and in animals,[10] but validity of their claims or a molecular mechanism of action in support of their claim still remain unexplored.

Homeopathic nosodes are prepared from microbes, secretions, discharges and tissues, and are quite frequently used in ultra-high dilutions like 30c in a variety of chronic ailments. Use of such nosodes in homeopathy dates back to 1833,[11] but their efficacy has seldom been systematically explored by conducting controlled experimental studies.[12]

Recently one of us (RS) developed an HIV nosode-30c (HIV 30c) from two AIDS-infected volunteers by adopting a widely accepted 15-step protocol.[13] In the current study, an indirect method has been used to test if HIV 30c might show any inhibitory effect on reverse transcriptase activity in cancer cell lines, particularly in lung cancer cells, A549. Allied to this, we made a specific attempt to evaluate regulatory effect, if any, of the HIV nosode on the proliferative activity of growth and division of cells, which are hallmarks of cancer cells, through deployment of various cytotoxicity assay methods including cell viability (MTT assay), cell morphology analysis, Annexin V/FITC assay, DAPI staining, drug−DNA interaction, DNA fragmentation, ROS generation, and MMP determination. Also included were proliferation and senescence assays and expression of pro-apoptotic (Bax, cytochrome c, caspase 3), and anti-apoptotic (Bcl2), telomerase reverse transcriptase (TERT) and topoisomerase II (Top II).

Materials and methods

Preparation and source of the nosode

HIV nosode preparation was done by one of us (RS) following the principal guidelines suggested by Samuel Hahnemann,[14] and approved by the Technical Committee of CCRH, New Delhi, Government of India[15,16] and the Homeopathic Guidelines of Drug Proving by the European Committee of Homeopathy through an elaborate 15-step safe method[13] from sera of two confirmed HIV-positive volunteers, by adopting the homeopathic procedure of potentization to obtain the 30c potency (dilution factor 10^{60}) using water/ethanol as solvent/vehicle after taking the statutory precautionary measures. The volunteers who donated blood samples were confirmed for their HIV types − type I or II. The donors of blood samples also tested negative for Hep B, Hep C, VDRL, Syphilis, etc. Co-infection positive samples, if any, were carefully discarded. Their sera were not included for preparation of the nosode. Viral load HIV-RNA quantification was done by the *in vitro* nucleic acid quantification method (COBAS Taqman) from the central laboratory "Metropolis" in Mumbai. Further, the 30c potencies of HIV nosodes from different batches were tested for viral copies, if any, by RT-PCR, which were found to be negative.[13]

ECH and CCRH guidelines are the only guidelines currently available for homeopathic drug provings, but are generally accepted. Ultradilute potentised homeopathic medicines may be devoid of any active material of the source; and are considered safe for human use, as per the well accepted Homeopathic Pharmacopeia of India (HPI), The Homeopathic Pharmacopeia of the United States (HPUS) (www.hpus.com), and similar other available pharmacopeia.

Cell culture

HeLa (cervical cancer), HepG2 (liver cancer), A549 (lung cancer) and normal liver hepatocytes, WRL68 cells were procured from National Centre for Cell Science (NCCS), Pune, India. All cell lines were maintained separately in DMEM containing 10% heat-inactivated FBS and 1% antibiotic mixture for cell culture in a humidified incubator with ambient O_2 level and 5% CO_2 level at 37°C.

Cell viability assay

For determination of cytotoxicity against cancer and normal cell lines, HeLa, A549, HepG2 and WRL-68 cells were treated with various concentrations of HIV 30c (0.5 μl−6 μl per 100 μl media) and incubated for ;24 h. MTT (3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide) solution (10 μmol/L) was then added to each well and incubated for 3 h at 37°C. Formazan crystals formed were dissolved in 100 μl acidic isopropanol and optical density (OD) was measured at 595 nm in an ELISA reader (Thermo scientific, USA).[17] On the basis of MTT assay results, the type of cancer cells showing best response, that is, the maximum number of cancer cells

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