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Evaluation of Anticancer Potential of the Unorthodox Homeopathic Nosode, HIV-30c, in A549 Cancer Cells: a Commentary on our Recently Published Research

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Abstract

A homeopathically prepared remedy, HIV nosode 30c was tested for its possible anticancer potential and therapeutic use against cancer as revealed from a recent study on A-549 lung cancer cells conducted in our lab by deploying certain molecular biology protocols that yielded some interesting and promising results. The implications of the study in regard to its possible practical application as an integrative therapy in oncology and on the understanding of the molecular mechanism of biological action of the ultra-highly diluted homeopathic drugs are pointed out.

Keywords: HIV 30c nosode; Therapeutic use; Anti-cancer potential; A549 lung cancer cell

Introduction

The Human Immunodeficiency Virus (HIV) is the causal organism for inducing the Acquired Immune Deficiency Syndrome (AIDS), commonly known as the AIDS disease. AIDS appeared for the first time in the 1980s. The retrovirus HIV infects human being and initially produces a brief period of influenza-like illness and then usually remains in the host without apparently showing any other typically detectable symptom(s) of its own. Soon it replicates their single stranded DNA to form double-stranded cDNA by reverse transcriptase activity and gets incorporated into the host genome without producing any more notable diagnostic symptom for a variable period of time [1]. Then, along with the transcription of the host genome, the incorporated viral DNA also starts transcribing the viral genes, the product of which begins to cause a progressive loss of CD4+ T-cells of the host, leading to almost total dysregulation of the host immune system. The host now becomes extremely susceptible to common infections, like tuberculosis, and other opportunistic infections and tumours, the most notable among them being the infection of the lung that leads to the condition known as "pneumocystis pneumonia", a disease associated with severe weight loss and skin lesions mainly caused by Kaposi's sarcoma, or by other AIDS-related conditions [2]. Kaposi's sarcoma had earlier been suspected to be produced by human herpesvirus 8 (HHV8), also known as Kaposi Sarcoma-associated Herpes Virus (KSHV) or KS agent in the 19th century [3]. but the viral origin of the disease was actually confirmed only in 1983 when the AIDS disease started spreading like an epidemic [4]. Therefore, apart from its primary effect on the immune system, the patients with the AIDS disease often end up showing severe secondary lung infection, caused by Kaposi sarcoma with timorous growth, which often turns cancerous in nature.

The AIDS disease rapidly spread in the 1980s killing thousands of patients and no proper treatment could be found at hand for a long time to combat this dreadful disease owing to unavailability of any proper anti-viral drug or specific strategy for effective control and treatment. As reverse transcriptase activity is extremely important for retro-virus replication for entry into the host genome, orthodox drugs like some reverse transcriptase inhibitors, or protease inhibitors were tested [5,6]. But while they showed their ability to interrupt the virus from replicating as expected, unfortunately because of their severe side-effects, these drugs had to be soon withdrawn or their use could no longer be recommended for AIDS.

Two major hallmarks of cancer cells are their acquired property of immortalization and uncontrolled cell division, among others. For continuous and rapid cell division, one pre-requisite is the continuous synthesis of the telomeres (chromosome ends) that determines the precise time when the chromosome is ready for the next division. Therefore, one of the other characteristic features of the cancer cells is their ability to drive the telomere synthesis faster to cope up with the urge of the cancer cells to divide and re-divide. Telomerase is the specific enzyme that helps in this activity

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of the telomere synthesis. For the divisional activity of the telomere zone of the chromosome, the Telom Erase Reverse Transcriptase (TERT) enzyme forms a part of the sub-unit which is activated and expressed more in order to help the rapid synthesis of the telomeres of chromosomes [7] to facilitate faster division. For the synthesis of the two-stranded cDNA structure from the single stranded viral RNA, the reverse transcriptase enzyme is also necessary. Therefore, anything that inhibits the expression of TERT gene, is significant not only for its direct role in inhibition of telomerase activity, but also for its effective anti-retroviral role in preventing cDNA formation and thereby incorporation into the host genome. Similarly, certain enzymes are critically needed for the DNA synthesis and replication process. Topoisomerase II (Top II) is one among them that can serve as a marker for the efficiency of perfect DNA replication process necessary for the cell division [8]. Therefore, stopping the ability of the DNA replication process should contribute towards restricting the process of cell division at a crucial stage, that has also been recognized as a legitimate target for all the anti-cancer drugs.

If and when, the cancer cells bypass all opposing forces of cell division, there is also an intrinsic mechanism to kill these errant cells by a mechanism of programmed cell death (apoptosis) or by necrosis or autophagy [9]. For inducing apoptosis, which is basically controlled by the ratio of the pro-apoptotic signals (like Bax, Caspase-3, Cytochrome c etc.) and anti-apoptotic signals (like Bcl2, TERT, Top II), these defiant dividing cancer cells are directed to the apoptotic pathway mainly through the action of the "gate-keeper" gene, the p53. Therefore, the study of relevant signalling pathway markers can give important clues about the molecular event/status if the cells are undergoing the apoptotic pathway. Any drug that can increase the apoptosis may be suggested as opposing the carcinogenic process.

Nosodes are highly diluted homoeopathically prepared drugs using biological materials such as diseased tissues, organisms, cultures (bacteria, fungi, and viruses), or parasites, or from decomposed products from humans or animals as the primary source material of the drug. In homeopathic practices, more than forty-five such nosodes have so far been in use since 1830 [10]. These nosodes are generally used in highly-diluted forms and known to be non-toxic in nature. Though clinical benefits of nosodes have often been experienced by homeopathic practitioners and patients, not many of them have been scientifically tested for their claimed efficacy [11-13]. HIV nosode 30c (henceforth to be called HIV-30) was developed from the sera of two serologically confirmed AIDS-infected volunteers as per 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 [17]. The standard homeopathic procedure of potentization elaborated in Khuda-Bukhsh [18]) was followed to obtain the 30c potency (the dilution factor being 10^{60}) using water/ethanol as solvent/vehicle after taking the statutory recommended precautionary measures. This dilution factor of 10^{60} in the potency 30c would suggest that even existence of a single molecule of the original nosode material is highly improbable in the drug. The HIV-30 was mixed with the media in three different doses, namely, the 50% lethal dose (LD50)(3.5ml/ 100 ml media) and two doses below the LD50 (at 3ml/100 ml media and 2.5 ml/100 ml media, respectively) for further experiments; Dose-1, -2 and -3 were designated from lowest to highest dose. The cells treated with succussed ethanol from the same stock with which the drug was prepared served as the control.

Although initial testing of efficacy of the HIV-30 was done on three different cancer cell lines- HeLa (cervical cancer), HepG2 (liver cancer), A 549 was (lung cancer) and on the normal liver hepatocytes, WRL68 cells (control) procured from National Centre for Cell Science (NCCS), Pune, India and 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, subsequently detailed analysis deploying various other specific protocols was carried out only on the liver cancer A549 cells, keeping WRL-68 normal liver cells as control.

Multiple routine standardized procedures used for authentic evaluation of anti-cancer potential, like cell viability (MTT) assay, cell morphological observation, apoptotic analysis, nuclear morphology analysis by DAPI staining, drug-DNA interaction analysis by circular dichroism spectroscopy, DNA fragmentation assay, determination of Reactive Oxygen Species (ROS) generation and accumulation, analysis of changes of Mitochondrial Membrane Potential (MMP), proliferation assay, migration assay, analysis of b-galactosidase associated senescence, analysis of expressions of proteins related to cytotoxicity by Western blot including analysis of expression of telomerase reverse transcriptase (TERT) and topoisomerse II (Top II) associated with DNA/cell replication, as may be found in the original paper [18] were considered for understanding the cellular and mechanistic principles behind the efficacy of the drug. Statistical support was provided wherever necessary. These parameters of study are the known indicators of the status of cell division, migration and metastasis, and also cell death. The effects of HIV 30 nosode in the A549 cells vis-à-vis WRL-68 cells were analyzed as compared to that of the other control arm, the succussed alcohol 30c. The overall results clearly established anticancer potential of HIV-30, and also demonstrated its ability to inhibit the expression of both TERT and Top II signal proteins. The favourable modulation of both these proteins would strongly suggest that HIV-30 had the ability to inhibit not only the cancer cell division principally through decrease in telomere synthesis, which is a pre-requisite for cell division, but also indicative of the possible role it could play in the prevention or slowing down of the formation of the double stranded cDNA from the single stranded retro-viral RNA.

Although how the microdoses of the homeopathic remedy HIV-30 could alter various pathological conditions is still not fully resolved. However, several aspects related to research outcome on efficacy and on the question of understanding how the miniscule dose of the ultra-highly diluted homeopathic remedy can produce regulatory influence through possible epigenetic modifications have been elaborately dealt with in several of our earlier publications [19-28] after the present author first proposed the hypothesis [29] in 1997 that the ultra-highly diluted homeopathic drugs possibly acted through regulation of gene expression by an effective intrinsic molecular mechanism by triggering a cascade of gene action in a regulatory manner. Recently this "gene regulatory hypothesis" is steadily gaining ground and has also been supported as the most plausible and correct hypothesis in explaining the molecular mechanism of the potentized homeopathic drugs in all living systems, both in plants and animals and both *in vivo* and *in vitro* conditions. This hypothesis has been supported by some other prominent workers in the allied field of research [30]. Further works in elucidating more precisely the mechanism and pathway(s) of action of different homeopathically diluted drugs have now become an exciting area of research.

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