Commentary

Modulation of TERT and Top II Activities by the Homeopathic Nosode, Hep C 30 in Demonstrating its Anticancer Potential against Hep G2 Liver Cancer Cells: A Commentary on one of our Published Research

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Summary

A homeopathic nosode, Hep C 30 was tested for its possible anticancer potential in three types of cancer cells and its therapeutic potential was tested more elaborately against liver cancer cells (Hep G2) by utilizing certain molecular biology protocols including the telomerase reverse transcriptase (TERT) and topoisomerase II (Top II) activities. In this commentary, a critical analysis has been made on the possible implications of the study in respect of its practical application in integrative therapy in oncology and also elucidation of possible molecular mechanism of its biological action of the ultra-highly diluted homeopathic drug – a hotly debated scientific issue.

Key Words: Hepatitis 30C Nosode; Therapeutic Use; Anti-Cancer Potential; Liver Cancer Cells

Liver cancer, scientifically termed as hepatocellular carcinoma (HCC), is the fifth most common human cancer, and the third most common cause for cancer death in the world [1]. One of the major causes of HCC is associated with chronic hepatitis C virus infection that causes secondary cirrhosis, and approximately one million deaths occur each year as a consequence of cirrhosis [2]. The incidence of HCC is further expected to increase in the next two decades, largely due to hepatitis C infection. Therefore, HCC has become a great health concern and efforts through various systems of medicine are constantly being made to combat liver cirrhosis as well as the consequent cancer, particularly because of the lack of a fully dependable drug.

Homeopathy is a holistic form of complementary medicine initiated and propagated by a German physician, Dr. Samuel Hahnemann. This medical practice treats a disease by the administration of miniscule doses of a remedy (often ultra-highly diluted and succussed or dynamized [3] that would in larger amount produce in a healthy person symptoms similar to those of the disease; this doctrine is better known as “similis similibus curentur” or “like cures like”. In other words, if Hepatitis C retrovirus can cause chronic infection of liver and produce liver cirrhosis and develop HCC, then according to this contention, it would be expected that ultra-highly diluted miniscule doses of homeopathic remedy prepared from serum of Hepatitis C virus infected person by homeopathic dilution and agitation (potentization or dynamization) method can combat or ameliorate symptoms of cirrhosis and HCC produced due to chronic Hepatitis C retrovirus infection. Though homeopathy is extremely controversial in some advanced countries, it is a popular mode of treatment in India (only second to the modern western medicines) and some neighbouring countries and also some European as well as third world countries. Unlike in some Western countries, it is recognized as a legal system under the ministry of AYUSH, Government of India, with all benefits that only the modern system of medicine enjoys in many advanced countries.

With this background scenario, the present commentary deals with some interesting results obtained in our controlled in vitro laboratory experimental study which had been published as a regular research article [4] in the Journal of Integrative Medicine (Publ. Science Press and Elsevier, Singapore). In this article, the efficacy of the nosode Hepatitis C 30C (henceforth to be mentioned as Hep C 30) prepared by Dr. Rajesh Shah [5,6] following the approved standard homeopathic process of drug preparation was given to our laboratory for the purpose of examining if it had anticancer effects against liver cancer cells (Hep G2 cells) in vitro by utilizing certain widely accepted molecular biology protocols maintaining suitable controls.

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In homeopathy, the potentized nosodes are mostly produced from ultra-highly diluted substances sourced from biological materials such as diseased tissues, organisms, cultures (bacteria, fungi and viruses), or parasites, or from decomposed products from humans or animals. More than 45 such nosodes have been known to be in use since 1830 with claimed clinical benefits [5,6], but only very few have so far been scientifically tested for their anticipated effects, particularly utilizing in vitro models [4,7,8].

In view of the ability of hepatitis virus to mainly target liver causing critical diseases like hepatitis, inflammation and cirrhosis of liver and malignancy, we were interested in examining if ultra-high dilutions (30C, diluted homeopathically by a factor of $10^{40}$ times) of the Hep C nosode (mother tincture of serum of Hep C retrovirus infected person) could demonstrate anticancer potential in vitro at the molecular level, as evidenced from favourable modulation of some scientifically accepted molecular biology protocols, particularly in the expression of telomerase (TERT) and topoisomerase II (Top II) enzymes. The roles of these two enzymes are considered very important and taken as specific markers, respectively, for the synthesis of telomere (a pre-condition for rapid cell divisional activity), and related to the uncoiling of intertwined DNA coils during DNA replication and transcription [9].

In cancer cells, for continuous and rapid cell division, one pre-requisite condition is the continuous synthesis of the telomeres (chromosome ends) that determines the precise time when the chromosome is ready for the next division. Therefore, cancer cells attain the characteristic feature of rapid telomere synthesis, so that they can divide and re-divide at a faster rate [9]. Telomerase is the specific enzyme that helps in this activity of the telomere synthesis. Thus, anything that can slow down the process of telomerase activity will also slow down the synthesis of telomere which in turn will mean an anti-cancer action. In the reported study, there was a clear down regulation of telomerase reverse transcriptase (TERT) activity after administration of the homeopathic nosode Hep C 30. Further, 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, which is necessary for their incorporation into the host genome.

Similarly, for rapid cell division, rapid DNA replication is also very important. For this, certain enzymes are critically needed. Topoisomerase II (Top II) is one of the enzymes directly related with the DNA replication process. Thus, if a drug can inhibit the synthesis of this enzyme, the replication process will get affected [10]. Therefore, most anti-cancer drugs are known to target this enzyme, and an effective inhibition of this enzyme will affect the DNA replication, and slow down the cell division. When the cancer cells bypass all opposing forces of cell division, other mechanisms come into play to stop the division by way of killing the errant cells- either by programmed cell death (apoptosis) or by necrosis or autophagy [11]. Apoptosis is induced by a shift towards increased ratio of the pro-apoptotic signals (like Bax, Caspase-3, Cytochrome c etc.) to anti-apoptotic signals (like Bcl2, TERT, Top II); as a result, the cancer cells are directed to the apoptotic pathway mainly through the action of the “gate-keeper” gene, the p53. Therefore, any drug that can help in inducing apoptosis may be suggested as opposing the carcinogenic process, which the results of this study clearly showed as the administration of the Hep C 30 nosode clearly triggered apoptosis.

In this study, other standard protocols used for authentic evaluation of anti-cancer potential, like cell viability (MTT) assay, cell morphological observation, 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 β-galactosidase associated senescence, etc. have also been used [4], which further supported anticancer potential of Hep C 30 nosode. These parameters are known indicators of the status of cell division, migration and metastasis, and also cell death. The effects of Hep C30 nosode in the Hep G2 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 Hep C 30 nosode. The most pertinent question asked by rationalists is about the dilution of the nosode used; Hep C 30 nosode was diluted by a factor of $10^{40}$, which takes the dilution to such an extent where it goes far beyond the Avogadro's limit. This would mean that theoretically speaking it is highly improbable that even a single molecule of the original nosode substance (obtained from serum of the carrier of Hepatitis C virus) could be present in this diluted remedy Hep C 30. This has a favorable aspect in that the chances of any contamination of the original dead virus/debris, if any, will also be remote in the ultra-highly diluted remedy because of the homeopathic dilution procedure of repeated agitations and step-wise dilutions in alcohol during the potentization process [5,6]. However, on the other hand, the nature of the actual medicine and its mechanism of action in such a high-dilution remedy become unclear and therefore need some scientific explanation. In some earlier studies, it was also revealed that the regulatory influence of the ultra-highly diluted homeopathic remedies could possibly be mediated through epigenetic modifications [12,13]. On the basis of ability of some ultra-highly diluted potentized homeopathic drugs showing protective (repair) effects against cytogenetic and enzymatic damage/dysfunction induced by radiation, chemical toxicants, etc. [14-20] it was hypothesized that the ultra-highly diluted homeopathic drugs possibly acted through regulation of gene expression by an effective intrinsic molecular mechanism involving a cascade of gene action. Recently, this proposition 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 by some other
prominent workers [21,22]. Thus, further explorative 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. More in-depth research in suitable animal models followed by human trial would be necessary to recommend its precise therapeutic use; however, at present it shows promise of being a candidate as a future drug, at least as a supportive medicine, in the treatment of liver cirrhosis or HCC.

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**References**