EFFECT OF HOMOEOPATHIC DRUGS IN CONTROLLING MULTIPLICATION OF HEPATITIS –B VIRUS

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Summary

The present study describes an *in vitro* effect of homoeopathic drugs on HBV-associated DNA-polymerase activity. A total number of twenty samples of seven Homoeopathic drugs specially prepared in different potencies in *distilled water* were tested. Twelve out of twenty samples of drugs showed an inhibitory effect on DNA-P activity. The extent of inhibition varied with dilutions and the drugs used. This study was presumed to be helpful in understanding the possible use of these drugs in anti-viral therapy for hepatitis –B infection. Also this inhibitive activity of the Homoeopathic drugs in micro-dilutions (High Potencies) explains the curative action of Homoeopathic medicines administered upon patients, and opens an entirely new area of research to explore the specificity of particular micro-dilutions (potencies) of different Homoeopathic medicines in achieving the optimum curative effects therefrom.

Introduction

Hepatitis B virus (HBV) is one of the major causes of viral hepatitis. Recent studies have demonstrated that HBV is not a cytopathic virus. Liver necrosis during HBV infection is caused by an apparent immunological mechanism where ongoing HBV replication plays a major role. Now it is assumed that arrest of HBV replication will be the best way of controlling HBV infection.

A few drugs such as interferone, adenine arabinoside (ARA-A) are already in use to check viral replication in hepatitis –B patients. However, non-availability and high costs of these drugs have limited their general use. There are some homoeopathic drugs which are commonly used for the treatment of viral hepatitis, though their exact role in HBV infection is not known.

In the present study we have attempted to evaluate the effect of some homoeopathic drugs on *HBV associated DNA-polymerase activity in vitro*. The study may be helpful in giving information about the role of these drugs on HBV-replication.

Materials And Methods

Sera samples positive from HBV-associated DNA polymerase were obtained from patients of hepatitis-B admitted to the wards of All India Institute of Medical Sciences, New Delhi. The diagnosis of hepatitis-B was confirmed clinically, serologically and histopathologically. These sera were preserved frozen at –70°C till used. Hepatitis-B markers in these sera were assayed as described by Fang et al.

Homoeopathic medicines indicated for Liver affections and Jaundice in Kent's repertory[4] were worked out and a few of these were picked up for pilot study Bry, Card-m., Acon, Chel, Lyc, Bell, Berb-vulg., Nat-s, Nux-v, Nit-ac., Cal-ph., Podo, Nux-m, and Iod. These medicines were got prepared from raw substances in distilled water, in the potency range, 1X, 2X, 3X, 6X, 9X, 12X, 30C and 200 C each from the Homoeopathic Pharamacopeia Laboratory.[5]. At no stage of preparation was alcohol used since alcohol inhibits the enzymatic activity. The drugs were then coded before they were used in in-vitro study.

DNA Poylmerase Assay

DNA Polymerase associated with HBV was assayed as described by Fang et al.[3] In brief, all sera were

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filtered through 0.45μm filter before testing for DNA-P activity. 25 μl of serum was mixed with 10 μl of each detergent Non-idet P-40 (10%) and B-mercapto ethanol (3%) in microfuge tube (1.5ml). After 30 secs. 100 μ of freshly prepared reaction mixture—1.6 mol/L tris (hydroxymethyl) in aminomethane, 0.4 mol/L $Mgcl_2$, 1.2 mol/L NH_4cl, 5m mol/L each of d-CTP, d-GTP, and D-TTP (Sigma Chem Co.) and 5 mol–L of triturated ATP ammonium salt (BARC Bombay) was added to each tube. The tubes were vortexed for 30 seconds and incubated at 3°C for 4 hours in shaking water bath. After incubation, 50 μl of reaction mixture was spotted on glass microfibre filters (2.4 cm diameter) pretreated with 200 μl of trichloroacetic acid (5%). The filters were washed three times with trichloroacetic acid for 15 minutes each. The final washing was given with methanol and acetone for 5 minutes each. The discs were dried at 37 °C and counted in a scintillation counter with 10 ml of scintillation counting fluid (ugPPO and 0.1 POPOP per litre of toluene). A sample was considered positive for DNA-P if its count/min was higher than the mean count/min of negative control plus three standard deviation (mean+s.d.).

Effects of Drugs on DNA-P Activity

Equal volumes of serum (0.5ml) positive for DNA-P and Homoeopathic drug solutions in various potencies were incubated at 3°C for 24 hrs. and then an aliquot of this mixture was assayed for DNA-P activity. Serum containing distilled water instead of drug was used as the control. Reduction in DNA-P activity in presence of drug was calculated in reference to the control activity.

Results And Discussion

As a pilot study a total number of twenty samples of seven drugs picked at random from the coded lot were used to observe their effect on DNA-P activity *in vitro*. Twelve of twenty samples exhibited an apparent inhibitory effect on DNA-P activity.

The following observations were made from the varying extents of inhibition produced by the drugs on the DNA-P activity;.

i. Twelve of the twenty samples produced inhibition of DNA-P activity to different levels, and
ii. Different potencies of the same drug inhibited the enzymatic activity to different degrees.
iii. Homoeoapthic medicines, in potency (dilutions of the magnitude of 200C as well), were observed to inhibit DNA-P activity.

HBV replication results in the continuous production of viral protiens that initiate immunological reactions causing liver damage. Therefore arrest of DNA-P activity is an essential step to control HBV-replication. In asymptomatic carriers of HBV, there is a low grade HBV replication which ultimately leads to liver necrosis and liver cancer. Similarly, in acute liver failure patients, HBV replication causes a massive cell necrosis, which in certain proportions of patients results in fulminant hepatic failure and thus sudden death. Thus in all these cases, HBV replication is an initiating factor and then depending on the host-immune response, it either leads to carrier state or severe liver disease. In case this replication is controlled, the disease may also be controlled. HBeAg, HBV-DNA-P and BVH-DNA are the serological markers of HBV replication. Out of them DNA and DNA-P are more sensitive markers to indicate HBV replication. In the present study we followed the DNA-P level in serum so as to investigate the effect of homoeopathic drugs on HBV-vinions and is involved in the replication of HBV-particles. Inhibition of DNA-P will stop replication. The anti-viral therapy involving the use of interferone and ARAA etc. is already in practice but the lack of availability and high cost of these drugs have made them unpopular. Although the mechanism involved in the arrest of HBV replication by these drugs and the homoeopathic drugs are difficult, the basic motive behind their use is same, i.e. to check HBV replication.

In the present study we have exploited the inhibition of DNA-P activity as a means of terminating HBV replication. It is worth noting here that this effect is purely an *in vitro* effect. Whether this stands true even for in-vivo effect it has to be confirmed.

The full range of Homoeopathic drugs indicated for liver affections are to be tried for eliciting their in-vitro DNA-P inhibitive action in various potencies. A study from the data so received shall be done to find out various Homoeopathic drugs that can inhibit the DNA-P activity or control the multiplication of Hepatitis-B virus. Further study is in progress.

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