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Establishment of an *in vitro* assay system for screening hepatitis C virus protease inhibitors using high performance liquid chromatography.

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The hepatitis C virus (HCV) genome contains the code for a conserved, serine-type protease, called NS3, for the processing of the non-structural protein region of the viral polyproteins. Furthermore, a related protein NS4A is an effector or cofactor of NS3 protease activity in the cleavage of NS3-4A, NS4A-4B, NS4B-5A and NS5A-5B junctions. To establish an *in vitro* assay system for the screening of those enzyme inhibitors that inhibit the protease NS3-4A, we prepared a maltose-binding protein-NS3-NS4A fusion protein and a synthetic peptide substrate that mimics the NS5A-5B junction. Cleavage of the synthetic peptide was analyzed by reversed- phase high performance liquid chromatography (HPLC). We showed that the enzymatic activity of the NS3-NS4A fusion protein was enhanced in comparison to the NS3 protein alone. The assay conditions for optimum NS3-4A protease activity were determined to be pH 7.6 and 37°C. In addition, we evaluated several protease inhibitors using the same HPLC assay system. The activity of HCV protease NS3-4A was inhibited by 2714.4 μM diisopropyl fluorophosphate, 270.8 μM N-tosyl-L-lysyl chloromethyl ketone, and 825.5 μM Chymostatin. Moreover, we proved novel compounds inhibited HCV protease at lower concentrations. The results of the present study indicated that the synthetic peptide substrate and HPLC assay system are suitable for studying HCV protease activity and may facilitate the development of anti-HCV therapeutic reagents.

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Purification, Utilization of Exogenous Template and Application of Reverse Transcriptase of DHBV Replicative Complexes, P. Tao, X.W.Shao, Institute of Medicinal Biotechnology CAMS, Beijing, P.R.China

Duck hepatitis B virus replicative complexes(DHBV RCs)was isolated from acute DHBV-infected duckling liver by three steps. The relative endogenous DNAP activity of the purified DHBV RCs was increased by 100 times. The specificity of DHBV RCs was identified by Dot-hybridization with DHBV DNA and Dot-EIA with DHBcAg. The utilization of exogenous template in RT reaction of DHBV RCs was determined by incorporation of 3HdTTP into poly(rA).$(dT)_{12-18}$. The results indicated that DHBV RCs could use the exogenous template and it was dependent on the concentration of template. The activity was about 40% of that of the endogenous DNAP. Pretreatment of DHBV RCs with nonionic detergent, high concentration of salt and low pH did not enhance the activity of DHBV RT. It seems that the permeability of DHBV RCs was not the reason for low utilization of exogenous template . We further observed the effects of regular components in fermentation media on activities of DHBV RCs RT. The DHBV RCs RT had been used as a target for screening anti-HBV antibiotics from actinomyces isolated from soil samples with positive rate around 0.4%.

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Natural Interferon-α Treatment in Chronic Hepatitis B(HB) with Pre-core Stop Codon Mutation

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Recent studies have identified HBV strains with mutant DNAs in the pre-C/C region in chronic HB who seroconverted to anti-HBe. However, there have been very few reports on the effectiveness of interferon(IFN)-α in chronic hepatitis with the mutants. We have treated 7 children varying 1 to 15 years with IFN-α. The mutation of pre C stop codon mutation was confirmed by molecular cloning and sequence in each patient. They received 0.1MU/kg of natural IFN-α every day for 2 weeks and 3 times a week for additional 6 weeks. A complete response was determined by the absence of serum DNA-polymerase and HBV DNA and normalization of ALT/ AST within 6 months after the end of therapy. As controls, 20 chronic HB with HBeAg(wild type virus) were treated in a same manner. Complete responses were found in 5 out of 7 patients (71%) with stop codon mutation, while 11 out of 20 patients (55%) with wild type viruses. Corresponding to the IFN therapy, the initial amount of pre-core mutant virus in sera ($1x10^{6-9}$copy/ml) decreased gradually and turnd to be negative within 6 months.Thus we conclude that natural IFN-α is effective for chronic hepatitis B with pre-core stop codon mutation.

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SERIAL CHAGES IN TITERS OF ANTIBODY TO HEPATITIS B ANTIGEN AFTER IMMUNIZATION OF INFANTS BORN TO MOTHER WITH HEPATITIS B E ANTIGEN

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To evaluate the long-term protection of hepatitis B vaccine (HB vaccine) in a high risk environment, we followed 50 infants born to mothers with hepatitis B e antigen. These infants were immunized with three-dose regimen of plasma-derived hepatitis B vaccine and were followed for up to 10 years. Two infants (4%) acquired hepatitis B virus (HBV)infection before one year of age. All the other 48 infants remained hepatitis B surface antigen negative during follow-up period. These data suggest that protection against significant HBV infection lasts for at least 10 years. We conclude that the long-term protection afforded by plasma- derived HB vaccine is satisfactory and that a routine further booster dose before 10 years of age is not necessary.