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In vitro evaluation of combination therapies against hepatitis B virus replication[1]

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Abstract

The HBV-producing human hepatoblastoma cell line, 2.2.15, has been shown to be an accurate model of chronic cellular viral infection and a predictive model of antiviral response for in vivo hepadnaviral infection. Our laboratory has utilized the 2.2.15 cell line in a standardized assay to examine treatment schemes which use combinations of clinically relevant nucleoside analogues, novel methods to deliver potentially useful nucleoside combinations, and treatments which simultaneously target different parts of the HBV replication pathway. For example, the combination of 3TC (lamivudine) with either alpha interferon or penciclovir significantly enhances the antiviral effectiveness of these agents against HBV replication in 2.2.15 cell culture.

Keywords: Hepatitis B virus; Alpha interferon; Combination therapies

1. Introduction

Hepatitis B Virus [HBV] is a causative agent of both acute and chronic hepatitis, a major etiologic factor of primary hepatocellular carcinoma, and a major global health problem with over 240 million estimated chronically-infected individuals. Although there are several promising antiviral agents currently in clinical trials, alpha interferon remains the only licensed drug for the treatment of chronic HBV infection.

Substantial problems have been encountered in clinical treatment trials of chronic HBV infection. Toxicity is usually associated with nucleoside analogue therapy, although in some recent clinical trials this problem has been apparently minimized. Long term therapy remains a necessity, and virus replication rapidly returns following the termination of drug treatment. Viral drug-resistance has not yet been encountered; however, clinical treatment trials with nucleosides have rarely lasted more than a few months. Combination therapies for HBV infection have the potential to address these, and other, issues by reducing the amount of drug required for efficacy, and by inducing a more complete and rapid shutdown of virus replication. This communication focuses on a preliminary assessment of combination treatments using three antiviral agents currently in promising clinical trials against chronic HBV replication.

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2. Materials and methods

Confluent cultures of 2.2.15 cells were maintained on 96 well-flat bottomed tissue culture plates in RPMI 1640 medium with 2% fetal bovine serum (Biofluids, Inc. Gaithersburg, MD), and were treated with 9 consecutive daily doses of test compounds as previously described (Korba and Gerin, 1992). An analysis of toxicity was performed using uptake of neutral red dye (Korba and Gerin, 1992). Antiviral effects were determined by the analysis of HBV virion DNA levels in culture medium using a blot hybridization method (Korba and Gerin, 1992). A total of 8 individual cultures, on two plates, were used for each of 6, serial, 3-fold dilutions of each drug or drug combination.

For the combination treatments, antiviral agents were mixed at ratios to approximately match their 90% effective concentrations [EC_{90}]. Thus, two drugs with equal potency would be mixed at a 1:1 molar ratio. Serial dilutions were then made of the drug mixtures, maintaining the same ratio for all dilutions. EC_{90} values were determined by linear regression analysis (Korba and Gerin, 1992) and analysis of synergism was performed using the COMBOSTAT program (Combostat Corp., Duluth, GA).

Recombinant human alpha B/D interferon [αIFN, Ciba Giegy, AG], was obtained from D..Gangemi (Clemson Univ.), and penciclovir [PCV] was obtained from G. Holan (CSIRO, Melbourne, Australia). 3TC (lamivudine, (−)β-L-2′,3′-dideoxy-3′-thiacytidine) was obtained from either R. Schinazi (Emory Univ.) for the αIFN experiments, or was purchased from Moravek Biochemicals, Inc. for the PCV experiments.

3. Results

We have previously demonstrated that a novel human recombinant interferon [IFN], a hybrid of the beta and delta classes of alpha-interferon, has antiviral activity against HBV replication in vitro in 2.2.15 cells and in vivo against the Woodchuck Hepatitis Virus in chronically-infected woodchucks (Korba et al., 1993). Although the antiviral mechanism of alpha interferon against HBV is not currently known, it is not believed to act primarily as a DNA polymerase inhibitor. Thus, a combination treatment with αIFN and a nucleotide analogue could potentially attack different parts of the HBV replication pathway.

A combination of 3TC (lamivudine), a promising nucleoside analogue currently in clinical trials against chronic HBV infection (Deinstag et al., 1994), and αIFN was used to treat 2.2.15 cells in culture. Low levels of 3TC substantially increased the effectiveness of αIFN (Table 1). The concentration of 3TC present in the αIFN/3TC combination mixtures at the EC_{90} levels (0.049 μM and 0.041 μM, respectively, for the 3000:1 and 10000:1 ratios) was significantly lower than the EC_{90} of 3TC used as a single antiviral agent in 2.2.15 cells (0.222 ± 0.024 μM) in this set of assays.

Penciclovir [PCV], in its orally available form (famciclovir, Famvir), is licensed for the treatment of Herpes virus infections. PCV has also been shown to be an effective inhibitor of the Duck Hepatitis B Virus [DHBV] in cultured primary duck hepatocytes and chronically-infected Peking Ducks, and HBV in 2.2.15 cell cultures (Shaw et al., 1994; Korba et al., in press). PCV is currently in clinical trials against chronic HBV infections. Similar to that observed for combinations with αIFN, 3TC substantially increased the potency of PCV (Table 1). The concentration of 3TC present in the PCV/3TC combination mixtures at the EC_{90} levels (0.011 μM and 0.007 μM, respectively, for the 3:1 and 10:1 ratios) was significantly lower than the EC_{90} of 3TC used as a single antiviral agent in 2.2.15 cells (0.241 ± 0.021 μM) in this set of assays.

The relative effectiveness of both the αIFN/3TC and PCV/3TC combinations appeared to be related to the level of 3TC present in the drug mixtures (Table 1). The presence of 3TC did not change the toxicity profile of either αIFN or PCV (Table 1). Analysis of the effectiveness of both the αIFN/3TC and PCV/3TC combinations by the COMBOSTAT combination evaluation program indicated that the antiviral effects observed for both sets combination treatments at the EC_{90}'s were synergistic when compared to the effectiveness of each antiviral alone (Table 1).

Table 1
Effect of antiviral treatments on HBV virion production by 2.2.15 cells

Treatment	CC_{50} (IU/ml)	EC_{90} (IU/ml)	S.I. (CC_{50}/EC_{90})	COMBOSTAT analysis (at EC_{90})
αIFN	60 000 ± 3400	989 ± 71	60	
αIFN + 3TC	68 000 ± 4900	146 ± 15	466	Synergism @ 3000:1
αIFN + 3TC	55 000 ± 4000	409 ± 45	135	Synergism @ 10000:1
Treatment	**CC_{50} (μM)**	**EC_{90} (μM)**	**S.I. (CC_{50}/EC_{90})**	**COMBOSTAT analysis (at EC_{90})**
PCV	404 ± 33	0.7 ± 0.05	577	
PCV +3TC	420 ± 30	0.03 ± 0.002	14006	Synergism @ 3:1
PCV +3TC	441 ± 39	0.07 ± 0.005	6300	Synergism @ 10:1

Values are presented as mean values (± standard deviation, S.D). Concentrations of αIFN are presented as International Units per milliliter ('IU/ml'). The relative concentrations of each drug in each of the combination treatments is indicated. For the PCV/3TC combinations, either 3-fold or 10-fold more PCV than 3TC ('3:1' or '10:1' ratios, respectively) was present in the mixture. For the αIFN/3TC combinations, either 3000 or 10000 IU/ml of aIFN was present for each 1.0 μM 3TC ('3000:1' or '10000:1' ratios, respectively). CC_{50}, 50% cytotoxic concentration, defined as the drug concentration at which a 50% inhibition of neutral red dye uptake (as compared to control [untreated] cultures) was observed. EC_{90}, 90% effective concentration, defined as the drug concentration at which a 10-fold reduction of HBV virion DNA levels (as compared to control [untreated] cultures) was observed. S.I., Selectivity Index. EC_{90} values are used for calculation of the S.I. since reductions of HBV DNA levels that are less than 3-fold are generally not statistically significant in this assay system (Korba and Gerin, 1992). Analysis of the effectiveness of the drug combination treatments by the COMBOSTAT combination evaluation program, at the EC_{90} value only, is presented in the last column. Synergistic effects may not have been observed at all dilutions of a combination treatment.

4. Discussion

We have provided preliminary data on in vitro evaluations of two potential clinically useful drug treatment combinations against HBV replication. Further studies on these and other potential combination therapies are currently in progress and are necessary in order to fully evaluate the results described here. The combination treatments used significantly increased antiviral effectiveness in a synergistic manner without an apparent change in toxicity profiles. The 2.2.15 cell line can be used to model potentially relevant drug combinations which may then be tested in experimental animal models for long term efficacy. Such studies have the potential to develop truly effective antiviral therapies against chronic HBV infection.

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