Institute for Hepatitis and Virus Research

Annual Progress Report: 2010 Formula Grant

Reporting Period

January 1, 2011 – June 30, 2011

Formula Grant Overview

Institute for Hepatitis and Virus Research received $16,013 in formula funds for the grant award period January 1, 2011 through December 31, 2011. Accomplishments for the reporting period are described below.

Research Project 1: Project Title and Purpose

Selective and Therapeutic Elimination of Cells that Produce Hepatitis B Virus - This is a project to develop an entirely new way to treat chronic hepatitis B virus (HBV) infection. It has been shown that long term antiviral suppression often beneficially results in a decline in the number of HBV infected cells. However, a major problem in current HBV medical management is that virus production often rebounds following cessation of therapy, or because of drug resistance. Our concept is to use a small molecule pharmaceutical that has already been shown to be safe in people to selectively eliminate the cells that produce HBV. With the source of HBV production eliminated, viremia and antigenemia will be stopped. This is an innovative approach to chronic HBV treatment that can play an important role in reducing morbidity and mortality associated with chronic HBV infection in Pennsylvania, the U.S., and worldwide.

Anticipated Duration of Project

1/1/2011 - 12/31/2011

Project Overview

The overall goal of this project is to advance a new method of managing chronic hepatitis B virus (HBV), with the ultimate goal of human use. Specifically, we will determine if drugs that selectively kill HBV infected cells in tissue culture, can be used safely and effectively in animals. We have determined that two drugs, a statin (atorvastatin) and a polyketide (meclocycline/demeclocycline), already known to be safe in people, are much more toxic to HBV producing cells in culture than they are to uninfected cells. We must now determine if there are better drugs in those classes for our purposes. This study will identify a compound with the best profile for selective cell elimination. The deliverable at the completion of this initial study will be the identification of a drug that is ready to be moved from tissue culture to future animal studies.

Specific Aim 1: Determination of the polyketide and statin that is most selective for toxicity to HBV producing cells, in tissue culture.
Objective 1A: Identification of the statin and polyketide with the best selectivity profile, in vitro. The best compound, chosen from a group of structurally related compounds, will be chosen for advancement based on ability to selectively eliminate HBV producing cells, in tissue culture.
Objective 1B: Validation of the statin and or polyketide with the best selectivity profile in secondary cell systems. The compound(s) chosen in Aim 1A will be validated for selective cell elimination, using diploid human liver cells and primary human liver cells, producing HBV.

In this study, we will perform a "lead optimization" and in vitro "dose" finding study with the lead drugs that we have identified from a screen of our safe compound library. This will allow us to determine which drug, atorvastin (or another statin) or demeclocycline/meclocycline (or another polyketide), is/are most promising as selective eliminators of HBV producing cells. We will use a cell culture assay to grow HBV producing and non-producing cells in vitro, co-cultivated with varying concentrations of our lead drugs. Cell viability will be determined by standard viability assays (MTT or vital staining, counting). Results will be confirmed in compounds showing selective toxicity for HBV producing cells through examination with an immunostaining assay. Pennsylvania-based undergraduate students will be recruited for summer internship positions to complete the goals of this project and provide enhanced training experiences for future young scientists.

Principal Investigator

Timothy Block, PhD
President, Director
Institute for Hepatitis and Virus Research
3805 Old Easton Road
Doylestown, PA 18902

Other Participating Researchers

Ju-Tao Guo, MD, Pamela Norton, PhD - employed by Drexel Institute for Biotechnology and Virology Research

Expected Research Outcomes and Benefits

There are approximately 2 million Americans living with chronic HBV, and research indicates that 15-40% of them will develop cirrhosis or liver cancer, which are both associated with high rates of mortality. Current antiviral therapy is associated with viral rebound in a significant proportion of patients. It is expected that this research project will result in the identification of a new method for treating chronic HBV, by profiling drugs that selectively eliminate HBV infected cells. Such a new treatment strategy could have important implications for the future management of chronic HBV. Thus, there is potential for reducing future morbidity and mortality rates associated with chronic HBV, including the prevention of liver cancer. Additionally, this project could reveal new chemical leads for future antiviral development to

augment existing antivirals and reduce emergence of resistant viruses. Outside of the realm of HBV, results of this project could have implications for the treatment of other chronic infections.

Finally, with the inclusion of summer internships, it is expected that this project will enhance training of Pennsylvanian undergraduate students, encouraging them to pursue careers in biomedical science. It is important to note that student recruitment will focus on minority students of Asian and Pacific Islander or African American descent, the two ethnicities in Pennsylvania that are disproportionately affected by both chronic hepatitis B and liver cancer.

Summary of Research Completed

The labs of Andrea Cuconatti, Ph.D., with HaiTau Guo, Ph.D. and Ju-Tau Guo, M.D., working with trainees John Rogowosky, Mathew Campagna, and Laura Dipaulohave have determined the following:

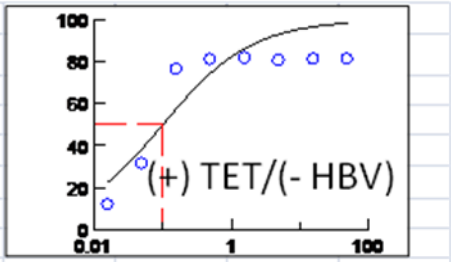
HBV producing cells, in culture, are consistently approximately 2-3 times more growth sensitive to NaK ATPaseion channel inhibitors, Oubain and Oleandrin, than are congenic cells that do not produce HBV. This is an interesting lead, since although the difference in sensitivity is small, it is reproducible, and suggests that HBV producing cells may require a level of NaK ATPAse activity greater than do uninfected cells. It also validates the approach. Another possibility is that the affects of Oubain and Oleandrin are due to “off target” effects. Two strategies will be taken from this point. The first will be to determine if other NaK ATPase inhbitors or other structurally related compounds have similar or, hopefully, enhanced, selective HBV cell producer killing affects against the cell systems used, to date, and other cell systems. If so, the mechanism of action and specific viral gene products associated with the selective cell killing will be pursued. The second strategy will be to screen a larger library compounds of selective HBV killing.

Results could also suggest that people who are chronic carriers of HBV might have selective sensitivity to drugs that are otherwise well tolerated by uninfected individuals.

Figure 1: Oubain appears to be selectively toxic to HBV producing cells. The liver tissue cancer cell derived line Huh7, was engineered such that it produces HBV when tetracycline is removed from the culture medium. In this way, the effect upon cell growth of various drugs, such as Oubain (shown here, in Figure 1A.) can be evaluated under conditions where the only apparent difference between the cells is tetracycline and the production of HBV. At 0.16 uM, Oubain has little effect upon the growth of these cells when HBV is not produced (tet on, less than 20% cytotoxicity). However, in the presence of even 0.016uM Oubain, as much as 60% of the cells are killed, when HBV is produced.

Drug, uM	%Via. Loss
50	81.24
16	81.12
5	80.52
1.6	81.61
0.5	81.00
0.16	76.44
0.05	31.35
0.016	11.75

Plate 1	Sample 3
Cmpd.	MS6 F3
CC_{50}	0.100
R^2	0.821



Drug, uM	%Via. Loss
50	78.15
16	78.01
5	78.57
1.6	79.54
0.5	78.84
0.16	75.78
0.05	70.08
0.016	57.13

Plate 2	Sample 3
Cmpd.	MS6 F3
CC_{50}	<0.016
R^2	0.617

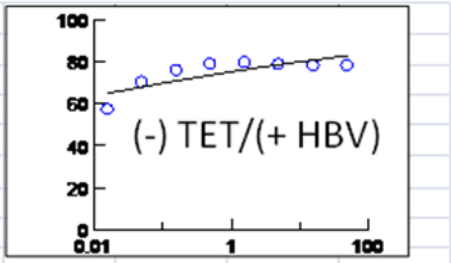
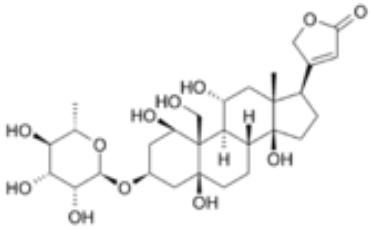



Figure 1A. Chemical structure of Oubain.