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Inhibition of Hepatitis B Virus Replication by Microalgal Extracts. BE Korba[1], P Behrens[2], KB Schwarz[3]. [1]Georgetown Univ., Div. of Molec. Virol. & Immunol., Rockville, MD, [2]Martek, Corp., Columbia, MD, [3]Johns Hopkins Univ. Hospital, Baltimore, MD.

Photosynthetic microalgae have been utilized as a source of several types of biological products including fluorescent dyes and complex polysaccharides. This underexplored class of organisms is now being investigated as a potential source of antiviral compounds against such agents as herpes simplex virus and HIV. We found 2 of 22 microalgal extracts (representing 22 genera from 3 algal divisions) to selectively inhibit Hepatitis B virus [HBV] replication in a chronically HBV-producing, human hepatoblastoma cell line, 2.2.15.; a methanol/methylene chloride extract (dissolved in 50% DMSO) of microalgae of the Chlorophyta division [MC9], and a 90% ethanol extract of microalgae from the Rhodophyta division [PS1]. MC9 (at 100 ug/ml) and PS1 (at 500 ug/ml) induced more than a 30-fold depression in the levels of extracellular HBV virion DNA and approximately a 90% loss of intracellular HBV DNA replication intermediates [RI] following 9 consecutive daily treatments. No toxicity was associated with these treatments as measured by neutral red dye uptake. PS1, which is enriched for polysaccharides, also inhibited the release of HBV surface antigen [HBsAg] and HBV e antigen [HBeAg] into the culture medium by 2.2.15 cells. HBsAg levels in culture medium declined over 10-fold by day 3 of treatment and became undetectable by day 9. HBeAg levels, which closely paralleled HBV virion levels, were not depressed by day 3, but were more than 30-fold lower than pretreatment levels by day 9. MC9, a varied mixture of compounds, did not affect the levels of either extracellular viral protein. Neither extract affected the level of HBV RNA, indicating that the effect of PS1 on HBV protein levels was post-transcriptional. Current efforts are focused upon further subfractionation and characterization of these extracts and more detailed examination into their mechanisms of action. Microalgae appear to represent a potential new source of naturally-occuring antiviral products against HBV.

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Identification and Metabolism Studies of The Active Stereo Isomer Responsible for Anti-Hepatitis B Virus from (±) 2',3'-Dideoxy-3'-Thiacytidine.

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(±) 2',3'-dideoxy-3'-thiacytidine (± SddC) was previously demonstrated to be a potent inhibitor of Hepatitis B virus (HBV) in our laboratory, although it was unclear which stereo isomer was active. After incubating these stereo isomers in the presence of human deoxycytidine deaminase, approximately 50% of the mixture was able to be deaminated. The stereo isomer resistant to the deamination was then subjected to anti-HBV study and found to be more active than a mixture of the two. Similar results were obtained using a mixture of their 5-fluoro analogues (± 5-FSddC). The active stereo isomer was identified as the (—) form, and (+) form was found to be responsible for the cytotoxicity associated with ± mixture. Unlike ddC, which is a potent inhibitor of mitochondrial DNA synthesis which results in a delayed toxicity such as peripheral neuropathy after long term usage, (—) SddC does not affect mitochondrial DNA synthesis. (—) SddC can be readily metabolized to its nucleotide triphosphate, (—) SddCTP, intracellularly. It was further demonstrated that (—) SddCTP was a potent inhibitor of HBV DNA synthesis. This study was supported by NIH grants CA44358 and AI25899.