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Studies On The Virus Encoded Protease, NS3, Of Hepatitis C Virus.

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Hepatitic C virus (HCV) is responsible for a large proportion of transfusion related hepatitis. However, the majority of HCV infections are acquired by other, as yet unknown, routes. HCV is an enveloped, positive strand RNA virus which is genetically related to the Flavi- and more especially the Pestiviruses. In common with viruses of these genera, the genomic RNA of HCV is translated from a single initiation site to produce a polyprotein comprising a contiguous array of the structural and non structure proteins of the virus. The polyprotein is cleaved into the mature viral proteins by at least two mechanisms. Most, if not all, of the cleavage events generating the structural proteins of the virus involve host cell encoded protease(s) (signalase(s)). However the majority of the cleavages which produce the non structural proteins involved in virus replication are carried out by a virus encoded protease. The non-structural protein NS3 contain sequence motifs typical of a serine protease, and by analogy with the Flavi- and Pestivirus family is the protease responsible for the maturation cleavage of the non structural proteins into their functionally active forms. Specific inhibitors of this essential protease function would, therefore, be potential anti-viral therapeutic agents. We have cloned and expressed portions of the non structural coding regions, including NS3, of a British isolate of HCV in order to study the protease and other enzymatic functions of the virus.

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Evaluation of the Potent Anti-HBV Agent (-)-5-Fluoro-2',3'-dideoxy-3'-thiacytidine (FTC) in a Novel *In Vivo* Model. L.Condreay, R.Jansen, T.Powdrill, L.Johnson, D.Selleseth, G.Painter, P.Furman, D.Averett, and N.Ellis. Burroughs Wellcome Co., Research Triangle Park, NC 27709, USA

A chimeric animal model using a HBV-producing cell line of human origin was developed to investigate the ability of FTC to affect HBV replication *in vivo*. Tumors were generated in NIH-*bg-nu-xid* mice by subcutaneous (s.c.) injection of a suspension of 10^7 hepatoblastoma 2.2.15 cells, which produce infectious HBV. Although s.c. tumors were easily visible in mice at one week post-injection, ultimate tumor size at harvest varied among individuals. Circulating levels of human alpha-fetoprotein (AFP) produced by the injected cells correlated with serum HBV levels and tumor weight. HBV replicative DNA intermediates (RI) were detected in tumors harvested from mice 4 weeks post-injection with 2.2.15 cells. To examine the antiviral efficacy of FTC, groups of tumor-bearing mice were dosed with 89, 18, 3.5, 0.86, and 0 mg/kg/day for 21 days in drinking water. Serum HBV levels in mice given the two highest doses were less than 10% of those observed in untreated mice. The lower doses of FTC also reduced virus levels but to a lesser extent. In contrast, serum AFP levels in control and drug-treated groups were similar. Southern blot analysis revealed that intracellular RI DNA levels in mice dosed with 89 and 18 mg/kg/day were less than 15 and 30%, respectively, of those detected in control mice. A slight effect on RI DNA levels was apparent even in the group dosed with 3.5 mg/kg/day. Interestingly, supercoiled DNA templates were also apparently reduced by the treatment with FTC. This effect was also observed on protein-free DNA isolated from FTC-treated 2.2.15 cells cultured *in vitro*. Therefore, orally administered FTC inhibits human hepadnavirus replication in tumorigenic cells of human origin at nontoxic doses in this murine model.