

CLEVUDINE IS EFFICIENTLY PHOSPHORYLATED TO THE ACTIVE TRIPHOSPHATE FORM IN HUMAN HEPATOCYTES

C. Niu ¹, E. Murakami ¹, M.J. Otto ¹, **P.A. Furman** ¹

¹ Pharmasset, Inc., Princeton, NJ, USA

Background: Clevudine is a nucleoside analog of the unnatural L-configuration that has potent anti-HBV activity in vitro and in vivo. In phase III studies in both HBeAg+ and HBeAg- patients, 30 mg clevudine given once daily demonstrated potent antiviral efficacy and significant biochemical improvement after 24 or 48 weeks of therapy. These effects were sustained in a significant portion of the patients when therapy was stopped. Here we describe the metabolism of clevudine in human hepatocytes.

Method: To study the metabolism of clevudine in human hepatocytes, cells were exposed to a range (0.5 μ M –100 μ M) of ³H-clevudine concentrations and extracted with 60% methanol. The mono-, di-, and triphosphate derivatives of clevudine were separated and quantified using high pressure liquid chromatography.

Results and Conclusions: The level of phosphorylation of clevudine was dependent upon exogenous drug concentration and exposure time. The major clevudine metabolite formed in human primary hepatocytes was the monophosphate. The intracellular concentrations of clevudine, clevudine-monophosphate, and clevudine-triphosphate increased in a dose dependent manner as the extracellular concentration of clevudine was increased, whereas the clevudine-diphosphate concentration remained constant. At a concentration of 10 μ M exogenously added clevudine, the concentration of clevudine triphosphate reached close to the maximum. When cells were exposed to extracellular concentrations of clevudine that approximated the human plasma C_{max} for the 30 mg dose, high concentrations of clevudine triphosphate (40-60 μ M) were detected. This intracellular concentration of clevudine triphosphate was approximately 300 to 500 fold higher than the inhibition constant (K_i = 0.12 μ M) reported for the HBV polymerase (Liu, et al. 1998). Exogenously added single nucleosides, thymidine, deoxycytidine, lamivudine, and emtricitabine, did not inhibit the phosphorylation of clevudine. Clevudine triphosphate persisted in cells after removal of the drug from the medium. The initial half-life of the triphosphate, following 24 hour incubation with clevudine and multiple wash-outs during the course of the experiment, was 16.5 h. The presence of clevudine in the medium, following a single wash-out, resulted in a longer persistence of intracellular clevudine triphosphate. These results are consistent with once daily dosing with clevudine in clinical trials.