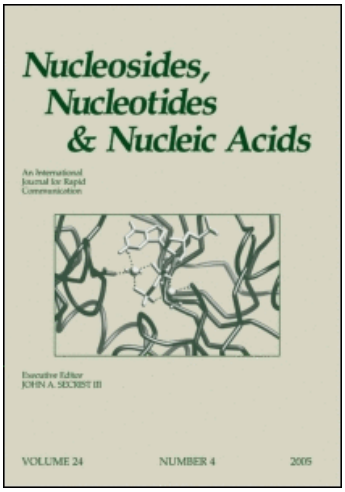

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Intracellular Metabolism of β-L-ddAMP-*bis*(tbutylSATE), a Potent Inhibitor of Hepatitis B Virus Replication

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INTRACELLULAR METABOLISM OF ß-L-ddAMP-Bis(tbutylSATE), A POTENT INHIBITOR OF HEPATITIS B VIRUS REPLICATION

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ABSTRACT: ß-L-ddAMP-bis(tbutylSATE) is a potent inhibitor of HBV replication with an EC_{50} = 0.1 µM. Following a 0-to72-hrs exposure of human hepatocytes to a 10 µM [2',3'-^{3}H] ß-L-ddAMP-bis(tbutylSATE), the pharmacologically active ß-L-ddATP was the predominant metabolite attaining a concentration of 268.53 ± 107.97 pmoles/10^6 cells at 2 hrs. In Hep-G2 cell, ß-L-ddATP accounted for 146.8 ± 29.8 pmoles/10^6 cells at 2 hrs with an half life of approximately 5.4 hrs. This study reveals that extensive intracellular concentrations of ß-L-ddATP after incubation of cells to the parent drug is accounting for its potent antiviral activity.

Introduction.

Chronic hepatitis B (HBV) is a major health problem and it is estimated that over 300 million people are chronically infected with hepatitis B virus[1]. Recently, we demonstrated that the pronucleotide ß-L-ddAMP-bis(tbutylSATE) is a very potent inhibitor of HBV replication in vitro in 2.2.15 cell-line with an EC_{50} value of 0.1 µM[2]. The present study was sought to investigate the intracellular pharmacology of ß-L-ddAMP-bis(tbutylSATE) in Hep-G2 cells and human hepatocytes in primary culture.

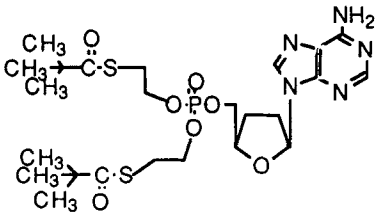

Figure 1: Structure of ß-L-ddAMP-bis(tbutylSATE)

Materials and methods.

ß-L-ddAMP-bis(tbutylSATE) was synthesized in our laboratories, and [^{3}H] ß-L-ddAMP-bis(tbutylSATE), (15.6 Ci/mmol) was obtained from Moravek Biochemical (Brea, CA) and were 99.9% pure as ascertained by the HPLC method described below.

After detachment of the adherent monolayer Hep G2 cells with trypsin-EDTA and three consecutive washes with medium, confluent Hep G2 (2 X 10^6 cells/ml) were resuspended in a final volume of 10 ml medium per time period and exposed to 10 µM [^{3}H] ß-L-ddAMP-bis(tbutylSATE) (SA 750 dpm/pmol). Cells were maintained at 37^{0}C under a 5% CO_2 atmosphere for specified time periods. At the selected time points cells were centrifuged then washed with cold PBS. ß-L-ddAMP-bis(tbutylSATE) and metabolites present iwere extracted by incubation overnight at -20^{0}C with 1 ml of 60% methanol. Hepatocytes were freshly isolated as previously described[3] and treated as described above. Samples were separated, dried and chromatographed to ensure purity then analyzed by LC-MS. Reversed-phase chromatography was performed with a Hypersil ODS 5 µm column.

Conclusion.

ß-L-ddAMP-bis(tbutylSATE) is extensively metabolized in human hepatocytes in primary culture and Hep-G2 cells leading to direct intracellular delivery of ß-L-ddAMP. Following a 0-to72-hrs exposure of human hepatocytes to a 10 µM [2',3'-^{3}H] ß-L-ddAMP-bis(tbutylSATE), the pharmacologically active ß-L-ddATP was the predominant metabolite attaining a concentration of 268.53 ± 107.97 pmoles/10^6 cells at 2 hrs. In Hep-G2 cell, the intracellular profile was different with ß-L-ddAMP being by far the predominant metabolite with concentrations as high as 356 pmoles/10^6 cells at 1 hr while ß-L-ddATP accounted for 146.8 ± 29.8 pmoles/10^6 cells at 2 hrs. In Hep-G2 cells ß-L-ddATP exhibited an elimination half-life (t1/2) of approximately 5.4 hrs with intracellular concentrations of 15.4 ± 2.08 pmoles/10^6 cells still measurable 24 hrs after the removal of the drug. This study reveals that extensive intracellular concentrations of ß-L-ddATP after incubation of cells to the parent drug along with its extensive half life account for its potent antiviral activity.

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