

This article was downloaded by:

On: 26 January 2011

Access details: Access Details: Free Access

Publisher Taylor & Francis

Informa Ltd Registered in England and Wales Registered Number: 1072954 Registered office: Mortimer House, 37-41 Mortimer Street, London W1T 3JH, UK



Nucleosides, Nucleotides and Nucleic Acids

Publication details, including instructions for authors and subscription information:

<http://www.informaworld.com/smpp/title~content=t713597286>

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

A. Faraj^a; L. Placidi^a; C. Perigaud^b; E. Cretton-scott^a; G. Gosselin^b; L. T. Martin^a; C. Pierra^b; R. F. Schinazi^c; J. L. Imbach^b; J. P. Sommadossi^a

^a Department of Pharmacology, University of Alabama, Birmingham, USA ^b Laboratoire de Chimie Bio-Organique, Universite de Montpellier II, France ^c Emory University School of Medicine/VA Medical Center, Decatur, GA, USA

To cite this Article Faraj, A. , Placidi, L. , Perigaud, C. , Cretton-scott, E. , Gosselin, G. , Martin, L. T. , Pierra, C. , Schinazi, R. F. , Imbach, J. L. and Sommadossi, J. P.(1999) 'Intracellular Metabolism of β -L-ddAMP-bis(tbutylSATE), a Potent Inhibitor of Hepatitis B Virus Replication', Nucleosides, Nucleotides and Nucleic Acids, 18: 4, 987 — 988

To link to this Article: DOI: 10.1080/15257779908041623

URL: <http://dx.doi.org/10.1080/15257779908041623>

PLEASE SCROLL DOWN FOR ARTICLE

Full terms and conditions of use: <http://www.informaworld.com/terms-and-conditions-of-access.pdf>

This article may be used for research, teaching and private study purposes. Any substantial or systematic reproduction, re-distribution, re-selling, loan or sub-licensing, systematic supply or distribution in any form to anyone is expressly forbidden.

The publisher does not give any warranty express or implied or make any representation that the contents will be complete or accurate or up to date. The accuracy of any instructions, formulae and drug doses should be independently verified with primary sources. The publisher shall not be liable for any loss, actions, claims, proceedings, demand or costs or damages whatsoever or howsoever caused arising directly or indirectly in connection with or arising out of the use of this material.

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

A. Faraj¹, L. Placidi¹, C. Perigaud², E. Cretton-Scott¹, G. Gosselin²,
L.T. Martin¹, C. Pierra², R.F. Schinazi³, J.L. Imbach², and J.P. Sommadossi^{1*}.

¹Department of Pharmacology, University of Alabama at Birmingham, USA;

²Laboratoire de Chimie Bio-Organique, Universite de Montpellier II, France;

³Emory University School of Medicine/VA Medical Center, Decatur, GA. USA.

ABSTRACT: β -L-ddAMP-bis(tbutylSATE) is a potent inhibitor of HBV replication with an $EC_{50} = 0.1 \mu M$. Following a 0-to72-hrs exposure of human hepatocytes to a $10 \mu M$ [$2',3'-^3H$] β -L-ddAMP-bis(tbutylSATE), the pharmacologically active β -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, β -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 β -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 virus¹. Recently, we demonstrated that the pronucleotide β -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 $0.1 \mu M$ ². The present study was sought to investigate the intracellular pharmacology of β -L-ddAMP-bis(tbutylSATE) in Hep-G2 cells and human hepatocytes in primary culture.

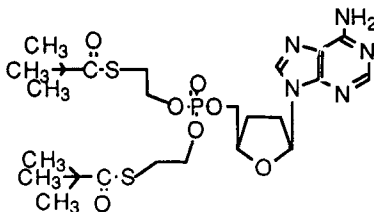


Figure 1: Structure of β -L-ddAMP-bis(tbutylSATE)

Materials and methods.

β -L-ddAMP-bis(tbutylSATE) was synthesized in our laboratories, and [^3H] β -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 (2×10^6 cells/ml) were resuspended in a final volume of 10 ml medium per time period and exposed to $10 \mu\text{M}$ [^3H] β -L-ddAMP-bis(tbutylSATE) (SA 750 dpm/pmol). Cells were maintained at 37°C under a 5% CO_2 atmosphere for specified time periods. At the selected time points cells were centrifuged then washed with cold PBS. β -L-ddAMP-bis(tbutylSATE) and metabolites present were extracted by incubation overnight at -20°C with 1 ml of 60% methanol. Hepatocytes were freshly isolated as previously described³ 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 $5 \mu\text{m}$ column.

Conclusion.

β -L-ddAMP-bis(tbutylSATE) is extensively metabolized in human hepatocytes in primary culture and Hep-G2 cells leading to direct intracellular delivery of β -L-ddAMP. Following a 0-to72-hrs exposure of human hepatocytes to a $10 \mu\text{M}$ [$2',3'\text{-}^3\text{H}$] β -L-ddAMP-bis(tbutylSATE), the pharmacologically active β -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 β -L-ddAMP being by far the predominant metabolite with concentrations as high as 356 pmoles/ 10^6 cells at 1 hr while β -L-ddATP accounted for 146.8 ± 29.8 pmoles/ 10^6 cells at 2 hrs. In Hep-G2 cells β -L-ddATP exhibited an elimination half-life ($t_{1/2}$) 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 β -L-ddATP after incubation of cells to the parent drug along with its extensive half life account for its potent antiviral activity.

REFERENCES

1. Hoofnagle, J.H., Shafritz, D.A. and Popper H. *Hepatology* 1987, 7, 758-763.
2. Loi, A.G., Faraj, A., Pierra, C, Gosselin, G., Imbach, J.L., Locarnini, S.A., Groman, E.V., Schinazi, R.F. and Sommadossi, J.P. Abstract 20, II International Conference on viral hepatitis, December 15-19, 1998, Kona, Hawaii, USA.
3. Placidi, L, Cretton-Scott, E., De Sousa, G. Rahmani, R., Placidi, M. and Sommadossi, J.P. *Cancer Res.* 1995, 55, 3036-3042.