ANTIViral ASSay DeVelOPment Anti-JCV Drugs

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ABSTRACT

Introduction: There are currently no JCV-specific therapies available for clinical use. This study evaluates viral large T antigen (LTA) as a potential target for drug development. LTA is a hexameric protein with a helicase activity that is powered by ATP binding and hydrolysis. The helicase activity and ATPase function is critical for viral replication and inhibition by small molecules would disrupt the viral life cycle.

Methods: Recombinant JCV LTA was produced in E. coli and used to make a plasmid from Dr. K. Khalili. ATPase activity was measured using the malachite green assay. The IC50 for ATP was determined by also multiple titrations of the protein and the assay optimized for ATPase activity using LDDN brain-associated compound library of 75,000 compounds. The compounds in the library were selected with filters to conform to Lipinski-type parameters but also to have the physicochemical properties to be more likely to cross the blood brain barrier.

Results: Five compounds showed non-competitive inhibition of ATPase with an IC50 ≤ 10 µM. Modest antiviral activity was demonstrated in an immunofluorescence assay for JCV VP1 expression in COS7 cells (EC50 15.1, 18.1, 20.0, 26.6, and 52.5 µM respectively). In the MTS96 and Cell TiterGlo assays was >100 µM for all compounds in COS7 as well as HEK293 cells. However, two compounds inhibited cell proliferation in culture with IC50 values of 42.9 and 34.2 µM respectively. These compounds inhibited viral replication in a real-time PCR assay at concentrations between 10 and 100 µM, but cell replication was also proportionally affected.

Conclusion: LTA is a valid target for discovery of anti-JCV drugs. The hits identified can be starting points for medicinal chemistry to improve potency & selectivity. Screening of more libraries could also be consistent to identify compounds that may be more potent with acceptable cytotoxicity.

INTRODUCTION

We hypothesize that JCV inhibitory and protective drugs can be discovered by screening chemical libraries for compounds that can inhibit the helicase machinery associated with JCV large T antigen (LTA). LTA is good target for drug discovery because (a) it is a key viral protein required for DNA replication, (b) it is well conserved across multiple viral strains, and (c) there is no homologous protein present in human cells. Compounds that inhibit the helicase activity of the protein will have an inhibitory effect on viral DNA synthesis.

The ATPase assay described here is quite suited for high throughput screening, since it is a ‘mix and go’ assay. ATPase activities of SV40 large tumor antigen. J Virol 38959.

The assay was then miniaturized to 384-well plate format and automated for high throughput screening. The LDDN brain-associated small molecule library of 75,000 compounds was used for HTS.

In the compounds in the library were selected with filters to conform to Lipinski-type parameters but also to have the physicochemical properties to be more likely to cross the blood brain barrier.

RESULTS

The screened compounds had a Ki value of ATPase in HEK293 cells for LDN0012723, LDN0015182, LDN0063710, LDN0060230 and LDN0065780 showed non inhibitory activity. The IC50 values for ATPase inhibition were 2.6, 18.1, 34.2, and 52.5 µM, respectively. The IC50 for ATPase inhibition was determined to be 2.6 µM, but cell replication was also proportionally affected.

CONCLUSIONS

LTA is a valid target for discovery of anti-JCV drugs. The hits identified are likely starting points for medicinal chemistry to improve potency and selectivity. Screening of additional chemical libraries could also be considered to identify chemical structures that may be more potent & acceptable cytotoxicity.

REFERENCE