ABSTRACT

6-Diazo-5-oxo-L-norleucine (DON), a potent glutamine antagonist, broadly blocks glutamine utilizing reactions critical for the synthesis of nucleic acids, amino acids, proteins, and the generation of alpha-ketoglutarate essential for energy metabolism. DON has shown robust efficacy in multiple preclinical cancer models and exploratory clinical studies. Although promising, development of DON was halted due to its dose-limiting gastrointestinal (GI) toxicity, as the GI system is highly dependent on glutamine utilization. Given DON’s promising efficacy, we developed novel tumor cell-targeted glutamine antagonists intended to circulate intact in plasma and be preferentially biotransformed to DON in tumor cells. Using a well-defined screening paradigm, we discovered compound 6, (isopropyl-2-(4-azamido-2-oxoaminomethyl) hexanamido)-6-diazo-5-oxohexanoate), that showed stability in plasma, liver and intestinal homogenates, yet was readily cleaved to DON in P493B human tumor cells. When directly compared to DON, compound 6 exhibited 55-fold enhanced P493B cell-to-plasma ratio. In a time-dependent study, compound 6 showed sustained DON delivery to P493B cells while maintaining minimal release in human plasma. Moreover, in a cell proliferation assay, compound 6 showed dose-dependent inhibition of P493B cell growth. Using plasma from CES1+/ mice, wild-type mice and human, we confirmed that compound 6 exhibited similar stability in CES1+/ mice and human plasma but not in wild-type mice plasma. We then performed pharmacokinetic evaluation in C78Bl/6 CES1-/ mice bearing flank murine EL4 tumors. These studies describe discovery of a tumor targeted glutamine antagonist. In addition, we introduced a murine model, that recapitulates human metabolism and can be broadly utilized in preclinical development. Future studies will investigate the dose dependent efficacy and utility of compound 6 in tumor bearing C78Bl/6 CES1-/ mice.

METHODS

Chemistry

In vitro plasma and tissue homogenate (liver and GI tissue) stability assays were performed as previously reported. Briefly, prodrugs were spiked in plasma or swine tissue homogenates and percent remaining at 1 h was determined via LC-MS/MS.

Following substantial dosing (1 mg/kg DON equivalent), compound 6 exhibited excellent pharmacokinetics with a 5-fold higher DON tumor exposure (AUC 3.1 mg hr/mL) versus plasma (1.1 mg hr/mL) and a 11-fold higher tumor exposure versus GI-tissues (tissue site, AUC = 0.45 mg hr/mL).

These studies describe discovery of a tumor targeted glutamine antagonist. In addition, we introduced a murine model, that recapitulates human metabolism and can be broadly utilized in preclinical development. Future studies will investigate the dose dependent efficacy and utility of compound 6 in tumor bearing C78Bl/6 CES1-/ mice.

RESULTS

Chemistry

In Vivo Metabolic Stability and Tumor-cell Partitioning of 6

In Vitro Bioactivation Mechanism, Stability with hRecombinant Enzymes and Tumor Cell Viability of 6

CONCLUSIONS

Successfully synthesized 6-acetylated lysine produg on DON’s amine of which prodrg 6 was the lead

6. showed stability in plasma, liver and intestinal homogenates, but was readily cleaved to DON in multiple tumor cells providing a 40-55 fold enhanced tumor cell-to-plasma ratio

The mechanism of bioactivation was shown to involve both cathepsin B and L.

Using CES1+ mice that recapitulated human metabolism, we showed 6 preferentially bioactivated in tumor affording 5- and 11-fold higher tumor exposure versus plasma and intestinal tissues, respectively

REFERENCES


RATIONALE

Mechanism of tumor cell targeting

Structure of Boc-Lys(Ac)-Puro