PRECLINICAL PHARMACOKINETIC STUDIES WITH S-HDAC-42 (NSC 736012), AN INHIBITOR OF HISTONE DEACETYLASE, BY LC-MS/MS

Hao Cheng[1], Zhongfa Liu[1], Samuel K. Kulp[1], Ching-Shih Chen[1], Joseph M. Covey[2], Kenneth K. Chan[3]

[1] College of Pharmacy, The Ohio State University, Columbus, OH 43210; [2] The National Cancer Institute, Rockville, MD 20852; [3] Colleges of Pharmacy and Medicine and Public Health, The Ohio State University, Columbus, OH 43210.

R, S-HDAC-42 (NSC 731438), an inhibitor of histone deacetylase, has been found to possess potent cytotoxicity in the NCI 60-cell line screen with a mean GI50 of 0.2 μM. The S-enantiomer of HDAC-42 (s-HDAC-42; NSC 736012) is the more potent isomer. The purpose of this study was to develop a highly sensitive LC-MS assay for the analysis of s-HDAC-42 in plasma and to characterize its pharmacokinetics in mice.

A liquid chromatograph-triple quadrupole mass spectrometer (LC-MS) with electrospray ionization (ESI) was used for quantification. Plasma (0.1 mL) was spiked with s-HDAC-42 and the internal standard, hesperetin and then extracted with ethyl acetate. The extracts were subjected to ESI LC-MS using a BetaBasic C8 column with isocratic elution. Positive ion selected reaction monitor (MRM) mode with ion transitions at m/z 313.2-133.2 for s-HDAC-42 and m/z 303.2-177.2 for hesperetin was used for quantification. The assay method was validated in mouse plasma. Pharmacokinetics (PK) of s-HDAC-42 in CD1F2 mice was studied following i.v. bolus administration at 20 mg/kg and p.o dose at 50 mg/kg.

The lower limit of quantification (LLOQ) for s-HDAC-42 was found to be 2 ng/ml (6 nM) in mouse plasma. Linearity was demonstrated between the LLOQ to 1000 ng/mL (3.2 μM). The within-day coefficients of variation (CVs) were found to be 14.7%, 9.5%, and 6.6% at 2, 5, 50 ng/mL (0.006, 0.016, 0.16 μM), the lower concentration range, while 6.1%, 6.0%, and 1.8% at 50, 500, 1000 ng/mL (0.16, 1.6, 3.2 μM), the higher range of concentration (n=6). The corresponding between-day CVs were found to be 8.8, 8.8, 0.6, 8.2, 1.4 and 0.6% , respectively. Plasma protein-binding of s-HDAC-42 was 95-98% at low μM range. Following an i.v. bolus dose at 20 mg/kg, plasma concentration reached ~49 μM then decreased to 0.02 μM at 48 hr. The data were found to fit to a two-compartment model with an initial and terminal half life of 0.38 hr and 10.1 hr, respectively. The AUC value was 43.7 μM.hr and the total body clearance was 1.47 L/h/kg at 20 mg/kg. s-HDAC-42 was absorbed rapidly following a 50 mg/kg p.o. dose and plasma concentration reached 14.7 mM at 0.17 h and also declined biexponentially with time. The oral bioavailability was found to be 27.4%.

In summary, an ESI LC-MS/MS method for s-HDAC-42 in plasma with low nM sensitivity has been developed and used to characterize the pharmacokinetics of s-HDAC-42 in mice. Oral absorption was rapid and bioavailability was moderate.

Supported byRAID #276 (C-S Chen) through NCI-N01-CM-52205. 2061/2600