A Quantitative Method for Determining Hyaluronan Content in Plasma by LC/MS/MS and Plasma HA as a Pharmacodynamic Marker for PEGylated-Hyaluronidase PH20 (PEGPH20) in a Phase 1b Trial for Pancreatic Ductal Adenocarcinoma

M. A. Printz1, B. Babson2, P. Selvam1, D. F. Beyerlein2, P. Jiang1, D. C. Maneval1, B. J. Sugarman1
1Hyaluronan (HA) is a non-sulfated linear glycosaminoglycan comprised of repeating disaccharide units of n-acetylglucosamine and glucuronic acid. HA is abundant in the extracellular matrix of tissues (e.g. skin, those undergoing rapid growth and development) and has been associated with wound repair and in aggressive malignancies. In solid tumors, abundant HA has been implicated in normally high interstitial fluid pressures (IFP), poor vascular perfusion, hypoxia, and is associated with poorer prognosis. PEGPH20, a systemically administered investigational hyaluronidase, degrades tumor-associated HA leading to an increase in tumor vascular perfusion, a reduction in IFP/hypoxia, and more efficient delivery of co-administered anti-cancer agents. Quantitation of plasma HA may provide a useful tool to characterize tumors that accumulate HA and may also be used as a pharmacodynamic (PD) marker for PEGPH20.

Repeating polymers of HA can vary in size from small oligosaccharide chains to high molecular weight forms (1-4 MDa). Ligand-binding and histochemical assays for HA tend to be semi-quantitative in nature due to the heterogeneity of the glycosaminoglycan, variable efficiencies of detection based on size, and the ability of small molecular weight HA to interfere in detection of the larger species. Early HPLC-based methods allowed for quantitative determination of HA content by total disaccharide content but were limited in sensitivity, making measurements of low endogenous serum/plasma concentrations difficult. We sought to improve the sensitivity of the HA disaccharide HPLC methods by coupling with MS/MS. The goal of the improved assay was to provide ng/mL sensitivity and the precision, accuracy, and dynamic range necessary for use as a PD marker and in PK/PD modeling for oncology trials investigating PEGPH20 in the treatment of solid tumors.

Methods

Human plasma samples containing K3-EDTA as the anticoagulant were assayed for total HA content by enzymatic digestion with chondroitinase ABC to liberate HA disaccharide. HA disaccharide was recovered by precipitation with ethanol and subsequently derivatized with 4-nitrobenzylhydroxylamine. A deuterium labeled 4-nitrobenzylhydroxylamine derivative of HA disaccharide was incorporated as an internal standard. Excess derivatizing reagent was removed by a solvent wash step and then the extracts were separated by HPLC using a Phenomenex Synergi MAX-RP column. The mobile phase was nebulized using heated nitrogen in a Z-spray source/interface set to electrospray negative ionization mode. The ionized compounds were detected using MS/MS. Quantitation of HA content in unknowns was determined by interpolation from calibration curves of known disaccharide standard quantities. The method was validated for use in human plasma for concentrations ranging from basal endogenous levels (~40 ng/mL) to approximately 400 μg/mL and allows for the accurate quantitation of HA independent of fragment size. Plasma samples from stage IV pancreatic ductal adenocarcinoma (PDA) patients enrolled in a Phase 1b clinical trial of PEGPH20 in combination with gemcitabine were evaluated for HA content at baseline and varying time points post-PEGPH20 administration to evaluate impact of systemic exposure to hyaluronidase on circulating concentrations of HA.

Results

Derivatization of HA disaccharide with 4-nitrobenzylhydroxylamine imparted greater hydrophobic retention and allowed chromatographic separation from chondroitin sulfate disaccharide, which has identical mass to HA. Moreover, derivatization allowed for the incorporation of a deuterium labeled 4-nitrobenzylhydroxylamine derivative of HA disaccharide as an internal standard. This feature of the assay was key to successful validation. Method validation studies demonstrated acceptable performance characteristics with intraday and interday precision and accuracies <15%, LLOQ intraday and interday precision and accuracies <20%, enzymatic digestion robustness precision and accuracies of <10%, appropriate selectivity/matrix interference characteristics and analyte carry-over <5%.

PDA patients in 3 cohorts were administered twice weekly doses of PEGPH20 at 1.0, 1.6, and 3.0 μg/kg in combination with gemcitabine. Plasma samples evaluated for HA catabolites by LC/MS/MS revealed a dose and time-dependent increase over the first 3 days. In general, HA concentrations continued to increase with time, reaching a steady-state after approximately 1 week of dosing, although inter-individual variation was observed. A dose-dependent increase in circulating plasma HA concentrations post-PEGPH20 administration provided evidence for systemic exposure to active hyaluronidase and supports the use of HA as a PD marker. The performance characteristics of the method allow for further use of data in subsequent PK/PD modeling. The measure of endogenous plasma HA is being investigated as an alternative prognostic marker for PDA.

Conclusion

A novel LC/MS/MS method for the quantitation of total HA content in plasma was developed and validated, which provides accurate quantitation of HA independent of chain length. This allows for determination of intact HA as well as oligosaccharide catabolites in human plasma samples.

This method has utility for measuring HA catabolites as a PD marker for PEGPH20-based treatment in patients with PDA.