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Appendix

Quantification of TXA in Plasma

TXA (American Regent Inc., Shirley, NY) was quantified in human plasma by LC-MS-MS similar to a previously reported procedure[13]. A 50 μ L sample of plasma was extracted in 200 μ L of ice-cold 80% methanol containing 500 ng/mL cis-4-aminocyclohexanecarboxylic acid internal standard (Sigma Aldrich, St. Louis, MO). After incubation at -20°C for 30 minutes and centrifugation at 14,000 rpm for 10 minutes at 4°C, the supernatant was transferred to a glass autosampler vial for analysis by LC-MS-MS.

LC-MS-MS analysis was performed on a SCIEX API-4000 triple quadrupole mass spectrometer (SCIEX, Framingham, MA) equipped with a Shimadzu Prominence HPLC (Shimadzu Corp., Kyoto, Japan). Chromatographic separation was obtained on a 3 μ m Hypersil ODS 50 \times 4.6 mm C18 column (ThermoFisher Scientific, Waltham, MA). Mobile phase A consisted of 2 mM ammonium acetate (pH 3.0) and mobile phase B consisted of 100% acetonitrile. After an initial 1.5 minute hold at 20% acetonitrile, the analyte and internal standard were eluted with a linear gradient over 2.5 minutes to 95% B, with a final hold at 95% B for 1 minute. The analyte and internal standard were measured by electrospray ionization in positive-mode with the following parameters: CUR, 20 psi; Gas 1, 50 psi; Gas 2, 48 psi; IS, 4500 V; TEM, 400°C; CAD, 8 psi. TXA and the internal standard were quantified by monitoring the transitions m/z 158.2 \rightarrow 95.1 and m/z 144.2 \rightarrow 126.1, respectively. An additional transition was monitored for each parent mass to confirm correct identification.

Data was processed using Analyst 1.6.1 software (SCIEX, Framingham, MA). TXA measurements were normalized to the signal of the internal standard and quantified on 1/x-weighted standard curves ranging from 100-5,000 ng/mL and 5,000-100,000 ng/mL. Intraday and interday assay performance was assessed and determined to be within acceptable limits for this assay (CV < 15%, Accuracy \pm 15%).

Appendix Figure 1 - Consolidated Standard of Reporting Trials (CONSORT) flow diagram showing patient progress for the first ten patients

