Project Title


Objectives/Goals

If I can create a measurable in vitro model that can aspirinize the platelets with varying amounts of aspirin, stimulate them with a coagulant, and measure the anti-coagulant activity, then I can determine an individual's customized Minimum Daily Aspirin Dosage.

Methods/Materials

Eight 4.5 cc Vacutainer tubes, with 0.5 cc of 3.2% Sodium Citrate, of blood were drawn from 11 healthy subjects.

Aspirin solutions were prepared by dissolving 500 mg of pure aspirin powder into 5 cc of 99% ethyl alcohol. This initial solution was further diluted in 1:9 ratio twice with 0.9% sodium chloride (normal saline), resulting making a 1 mg aspirin solution in 1% aqueous alcohol.

Varying amounts of aspirin were added to tube 1 to 8: Tube 1, the control, received no aspirin. Tube 2 received 2.5 µg. Tube 3 received 5 µg (10 mg daily or 40 mg saturation). Tube 4 received 7.5 µg (15 mg daily or 60 mg saturation). Tube 5 received 10 µg. Tube 6 received 20 µg. Tube 7 received 30 µg. Tube 8 received 40 µg. Tubes were left for two hours at room temperature to aspirinize platelets.

0.5 cc of aspirinized blood was mixed with 0.5 cc of normal saline in a cuvette. The cuvette was placed into a Chrono-Log Whole Blood Aggregometer with a plastic-coated magnetic stir-bar. It was warmed to 37o C and a straight baseline was established. Then, 10 microL Arachidonic Acid were added to stimulate platelets aggregation. The final concentration of Arachidonic Acid was 0.5 mM. As the platelets aggregated, they formed a non-conductive barrier between the electrodes of the probe, which decreased conductivity.

Precautions: Blood was drawn and handled using universal precautions by Dr. Koh.

Results

My results showed that it was indeed possible to create an in vitro model to determine the MDAD for each of the tested individuals, 8 female and 3 male. The MDADs ranged from 10mg to 80mg. The resulting MDADs for the subjects 1 to 11 were: 20 mg, 40 mg, 20 mg, 10 mg, 10 mg, 60 mg, 80 mg, 60 mg, 40 mg, 40 mg, 40 mg, respectively.

Conclusions/Discussion

My hypothesis was supported. I was able to design a measurable in vitro model that demonstrated the anticoagulant activities of varying levels of aspirin, and determine individual MDAD for anti-coagulant purposes. The MDAD for anti-coagulant purpose is truly individual.