**Determination of Recombinant Tissue Plasminogen Activator (tPA) Concentration for In Vitro Induction of Hyperfibrinolysis**

Rotational Thromboelastometry (ROTEM®) was utilized to compare the ability of both intravenous (IV) and intramuscular (IM) tranexamic acid (TXA) to reverse hyperfibrinolysis in this active hemorrhage model. In order to do this, an in vitro model was created. This was chosen over an in vivo model because of the prohibitive cost of recombinant tissue plasminogen activator (tPA) at the large doses required to induce hyperfibrinolysis in swine. Furthermore, the hemorrhage was completed in a controlled fashion through an arterial catheter and therefore would not be influenced by alterations in coagulation parameters. In vitro models of hyperfibrinolysis have been utilized in swine (1), but the concentration of tPA required to reliably induce hyperfibrinolysis in a Yorkshire cross swine had not been determined. Serial dilutions of tPA had been used to determine required concentrations in other animals by Fletcher, et al. (2,3). Using these examples, a pilot study was performed to answer this question.

tPA was purchased from Genentech (San Francisco, CA) as 50 mg lyophilized Alteplase (29 million IU). The powder was reconstituted in 50 mL of sterile water to create a 1 mg/mL solution. 10 μL was removed and added to 90 μL of dH20 for a new concentration of 100 μg/mL, and the remaining 490 μL was immediately stored at 2-8OC for future use. Serial dilutions were then performed with porcine whole blood to create the following final concentrations: 0.5, 1.0, 1.5, 1.72 (=1000 IU), 2.0, 2.5, 3.0, and 3.5 μg/mL. For example, to create the 0.5 μg/mL sample, 5 μL of the 100 μg/mL tPA solution was added 995 μL of whole blood. To create the 1.0 μg/mL tPA solution, 10 μL of the tPA solution was added to 990 μL of whole blood, and so on. 300 μL of these samples (porcine whole blood + tPA) was then mixed with 20 μL Startem and 20 μL Extem and added to the ROTEM® plate. The assay was run until LI30 was achieved. The concentration for which hyperfibrinolysis was reliably corrected was 3.0 μg/mL. This was the concentration chosen for use in the larger experiment.

**References**

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