Rotational Thromboelastometry
Applied to Preclinical Models: Minipig Acute Radiation Syndrome and Ex-Vivo Pharmacological Investigations

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ABSTRACT

Drug development relies on preclinical models for early stage efficacy and safety investigations. Rotational thromboelastometry is widely used in humans for diagnosis but limited data are available in preclinical drug development. We report rotational thromboelastometry data in a minipig model of acute radiation syndrome (ARS) and the application of this technology to detect drug-induced alterations in platelet (PLT) response, clot formation and thrombus lysis ex-vivo. Platelet rich plasma (PRP) from minipigs exposed to hemi-body radiation (14 and 16 Gy) was evaluated up to 60 days post-radiation. For ex-vivo tests, whole blood was obtained from cynomolgus monkeys and humans. Rising concentrations of inhibitors, such as heparin, abciximab, methotrexate, acetylsalicylic acid, and platelet activator, such as collagen and ADP were added to the blood samples, ex-vivo. Viscoelastic properties of the clot formation were assessed by thromboelastometry (ROTEM™), for 1.5 hours after recalcification of citrated whole blood with calcium chloride (Star-TEM reagent). Radiation-induced thrombocytopenia was associated with minimal changes to thromboelastometry with values that remained comparable to baseline throughout the post-radiation period. Ex-vivo PLT response was stable for 2 hours post-collection. Heparin completely inhibited clot formation at 1 unit/mL while at 0.1 unit/mL, clotting time (CT) was extended by 148%, clot formation time (CFT) increased by 454% and maximum clot firmness (MCF) was reduced by 32%. Abciximab (0.005-0.0075 mg/mL) caused dose-dependent ex-vivo inhibition in humans and monkeys. Methotrexate (0.004-0.0125 mg/mL) increased CT and decreased MCF. As previously reported, acetylsalicylic acid (0.1 mg/mL, 1 mg/mL, 10 mg/mL) did not significantly affect rotational thromboelastometry. With rising collagen concentrations, MCF decreased by -35%, -31% and -36% and increased MCF. Signs of saturation were observed at higher collagen concentrations. ADP (5, 10 and 20 µM) reduced CT and caused a dose-dependent increase in MCF. This study reports the use of rotational thromboelastography in ex-vivo and ex-vivo preclinical models relevant to drug development. Further investigations will be needed to confirm the translational potential of this technology in non-clinical drug development models. This work was funded by BARDA (contract #: HHS010201100030I).

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