THE PORCINE ABATTOIR BLOOD MODEL – VALIDATION OF HEMOCOMPATIBILITY BLOOD PARAMETERS FOR IN-VITRO TESTING

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Introduction

The hemocompatibility remains the major limitation of blood-contacting medical devices. While the evaluation and prediction of flow-related hemolysis can be done either by computational modeling or in-vitro blood experiments, thrombogenicity can hardly be evaluated in vitro and is therefore most often tackled in animal experiments for the first time [1, 2]. This is ethically questionable, expensive, and inefficient for device developing. Therefore, standardized in-vitro test methods for assessing the thrombogenicity of medical devices are required. However, this is currently failing due to required blood volumes > 450 mL, which exceed a human blood donation, and due to the absence of adequate analog fluids. Animal abattoir blood would overcome these limitations but is supposed to be not suitable for medical device thrombogenicity testing due to a lack of comparability to human blood and possible pre-activation from the slaughtering process.
To this end, we investigated a complete DIN EN ISO 10993-4 conform set of hemocompatibility parameters from human donors versus porcine abattoir blood.

Methods

Porcine blood was collected directly at the slaughterhouse after a cut in the jugular vein of the animals. Blood from one animal was separated in two blood bottles and immediately anticoagulated with 2000 IU/L enoxaparin (Clexane) and sodium citrate, (3.13 %, ratio 1:10) respectively. Human blood was collected at the Medical Center of the RWTH Aachen University (EK 23-092) and divided into two separate portions anticoagulated as the porcine blood.
After arrival at the lab, blood count (Idexx ProCyte Dx), blood gas analysis (ABL 800 flex), thromboelastometry (ROTEM®delta), fluorescence activated cell sorting (BD FACS Canto II) with CD61, CD62P, CD45 markers and impedance aggregometry (Multiplate® analyser) were measured in whole blood from both species. Plasma samples were frozen at -80 °C for further ELISA analysis. The ELISA test comprises the analysis of plasma parameters including serotonin, adrenalin, cortisol, PMN Elastase, soluble C5b-9, C3a, β-thromboglobulin, and thrombin-antithrombin-complex.

Results

Blood values of both species were within the ranges of the respective normal values, independent of the way of blood withdrawal. Porcine and human blood platelet activation (CD61-62P) and platelet-leucocyte aggregates (PLAs, CD62-45) measured by FACS (CD61-62P and CD62-45) showed significant differences ($p<0.05$) in the comparison of pre- and post-activation values. While CD61-62P revealed a minimum of 90 % activation for human blood, only around 56 % were achieved by porcine blood. Similarly, CD62-45 activation was about 20 % less for porcine blood compared to human blood (Figure 1).

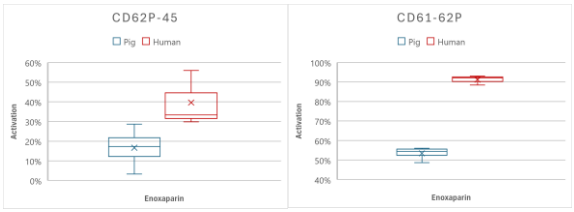

Figure 1: CD62-45 and CD61-62P activation of pig and human blood anticoagulated with enoxaparin.

The aggregometry with collagen activator presented different intra-species values depending on the anticoagulant. Interestingly, and in contrast to reports from literature [3], TRAP activation seemed to be functional for porcine blood as well.

Discussion

Porcine blood is still functional after blood collection at the slaughterhouse. Although presenting some decreased platelet activation potential, coagulation is still intact and generally useful for hemocompatibility testing according to ISO 10993-4. This is an important step towards medical device in-vitro testing with blood volumes >450 mL.

References

1. Sarode, et al, Expert Rev. Med. Devices, 16(7), 603-616, 2019.
2. DIN EN ISO 10993-4, 2017.
3. Heringer et al., PLOS ONE, 14(8), e0222010, 2019.

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