REVOLUTIONIZING BIOMEDICAL RESEARCH: INTEGRATING CUTTING-EDGE AI/ML TO UNLEASH INNOVATION IN DRUG DISCOVERY AND THERAPEUTICS DEVELOPMENT

Coupling Hemolysis Predictor Algorithms With Real-world Data For A Defibrinated Human Blood Hemolysis Assay For The Development Of Novel Antimicrobial Peptide Therapeutics To Target MDROs

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Background: The escalation of multi-drug resistant organisms (MDROs), intensified by the limited availability of new classes of antibiotics, calls for alternative solutions to be developed. Hemolytic testing of antimicrobial peptides (AMPs), or other drugs, is an important step in the development of novel therapeutics but is costly, labor intensive, and time consuming. Many computational algorithms claim to accurately predict the hemolytic potential of AMP sequences. In silico computational predictors offer an enticing alternative, possibly providing an initial screening of AMPs for hemolytic activity based on their primary sequence and could rapidly down-select successful from non-successful AMPs, excluding toxic sequences early in the AMP design and testing process.

Purpose and Objective: Accurately determining the hemolytic activity using human red blood cells (RBCs) will allow for a robust calculation of the therapeutic index of the candidate AMPs, a critical measure in their pre-clinical development. Using machine learning algorithms could potentially reduce obstacles to pre-clinical development of our lead candidates and could be a time-saving strategy to predict biological activities from primary sequences.

Rationale: Increased understanding of the parameters that contribute to both antimicrobial activity and toxicity/hemolysis could greatly improve our ability to design safe and effective AMPs and drive progress in the field. The limited reliability of existing AMP toxicity prediction algorithms remains a key obstacle to their widespread use in high-throughput programs for new AMP generation. Experimental determination of hemolytic activity against a relevant RBCs remains the gold standard for accurate determination of the potential for hemolysis for a given AMP or drug.

Methods: De-identified gender pooled blood was defibrinated, a process involving the removal of fibrin from the blood. Hemolysis assays were performed, and the percent hemolysis was calculated.

Preliminary Results: Starting with computational predictors such as HemoPred, ToxinPred, HAPPENN, and HemoPI, we calculated the theoretical hemolytic activity of a small pool of AMPs. Our observation of high hemolytic activity of LL-37 peptide against human RBCs that were collected in EDTA, in contrast to the expected hemolytic activity (both computational prediction and prior experimental data) led us to question both the computational approaches and the experimental approaches. We developed a more robust, accurate and simple hemolysis assay using defibrinated human blood. We found significant differences between the sensitivity of defibrinated RBCs and EDTA treated RBCs. We compared our real time results head-to-head with the computational predictors.

Preliminary Conclusions: We conclude that the computational predictors are almost all highly inaccurate. We introduce a standardized, more accurate protocol for experimentally assessing hemolytic activity using defibrinated human RBCs. Standardization is essential to reconcile inter-study variations and improve the reliability/reproducibility of AMP (or other drug) hemolytic activity assessments between laboratories. Our defibrinated human blood protocol presents a robust, highly relevant, and replicable solution.

Impact: The use of computational predictors combined with this standardized method of hemolysis aligns with the DTRA JSTO mission to enhance the Joint Force's capabilities by improving the reliability and reproducibility of hemolytic assessments for the development of AMPs as potential novel therapeutics; thus, potentially improving the warfighter health and readiness.

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