Innovative therapeutic approach based on nucleic acid aptamers for the treatment of sepsis-induced disseminated intravascular coagulation

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Résumé

Context & Hypothesis: Septic shock is complicated in 30% of cases by disseminated intravascular coagulation (DIC), linked to excessive activation of the coagulation cascade and fibrinolytic insufficiency. We hypothesize that targeted treatment aimed at simultaneously modulating the procoagulant response and restoring fibrinolytic balance in patients with septic DIC could reduce disseminated microthrombi, thus contributing to limiting organ dysfunction and reducing the associated mortality.
Objective. My thesis work aims to develop an innovative strategy based on the use of aptamers to vectorize microvesicles at the level of disseminated microthrombi. Aptamers are short sequences of oligonucleotides with high affinity and specificity for their targets, with many advantages over antibodies, such as their chemical synthesis with high reproducibility, and their less restrictive storage conditions. The proposed strategy consists of: (i) fibrinolytic molecules-enriched microvesicles derived from mesenchymal stem cells, aimed at restoring local fibrinolytic activity; (ii) an anticoagulant aptamer conjugated to a lipophilic molecule capable of anchoring in the membrane of fibrinolytic microvesicles, allowing the vectorization of microvesicles to microthrombi.

Method: The stability of the aptamer was evaluated on polyacrylamide gel after 72h incubation in plasma from septic patients. The coagulation parameters (thrombin, activated partial thromboplastin and prothrombin times) were measured by a haemostasis analyzer within 4 hours of blood sampling. The anchoring of aptamers to cells and microvesicles was confirmed respectively by flow cytometry and by the anticoagulant activity of the aptamer once anchored, particularly by inhibiting thrombin generation. Microvesicle characterization was performed with Zetasizer® to determine the Zeta potential, the diameter and microvesicle concentration was obtained by nanoparticle tracking analysis, and the amount of surface protein was determined at 280 nm with Nanodrop®. The microvesicle fibrinolytic activity was evaluated by monitoring plasmin generation by its chromogenic substrate.

Results: We first characterized the anticoagulant aptamer. Its half-life is 24 hours (n=3) in plasma from septic patients. The anticoagulant aptamer extends thrombin and activated partial thromboplastin times to 0.098 ± 0.020 s/nM and 0.086 ± 0.013 s/nM (n=3), respectively, and inhibit up to 90% thrombin generation (n=3). The tested aptamer concentration

*Intervenant

range (0.05–10 μM) enabled anchoring of the aptamer to 100% of living cells. Half-saturation of cells is 1μM aptamer per 500,000 cells (n=3). By comparing cell and microvesicle surfaces, this concentration allowed to determine the quantity of aptamer to be used for conjugation to microvesicles. After demonstrating the anchoring of aptamer in microvesicles by the ability of aptamer to inhibit thrombin generation (n=4), we showed the dual *in vitro* effect of aptamer-enriched microvesicles: anticoagulant and profibrinolytic activities without impacting the diameter of the microvesicles (n=3). The efficacy and tolerance of the *in vivo* strategy are ongoing to be evaluated in a murine model of septic DIC.
Conclusion: The innovative dual strategy based on the use of aptamers anchored to microvesicles appears to be a promising targeted therapeutic in septic DIC.

Mots-Clés: Sepsis, Disseminated intravascular coagulation, Aptamers, Coagulation, Fibrinolysis