POLYMERIC ANTICOAGULANTS BASED ON POLY(2-(ACRYL-AMIDO)-2-METHYLPROPANE-SULFONIC ACID) BLOCK POLYMERS

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Introduction

Maintaining equilibrium between two opposite processes, i.e. blood coagulation and fibrinolysis, is essential for proper functioning of the body. Any deviation from this state is potentially life-threatening. On one hand, insufficient blood coagulation may result in haemorrhage and dangerous blood loss, while on the other hand excessive blood coagulation may be the reason of clot formation within the blood vessels leading to more or less blocked blood flow and severe pathologies such as ischemic stroke or infarct of an organ.

The need of lowering excessive blood coagulation resulted in the advent of an important class of drugs – anticoagulants. Unfortunately, many of anticoagulants currently applied in clinics do not possess an antidote, e.g. low-molecular weight heparins (LMWHs) or fondaparinux.

Previously, we have obtained a heparin binding copolymer (HBC) which inhibits heparin [1]. Conversely, in this study we have synthesized and investigated di- and triblock polymers which show anticoagulative properties and therefore are potential anticoagulants. Importantly these polymers form polyelectrolyte complexes (PECs) with HBC, which therefore may constitute an antidote for these polymers.

Materials

4-Cyanopentanoic acid dithiobenzoate (CPD) was synthesized according to the method reported by McCormick and coworkers[26]. α,ω-Bis-hydroxy poly(ethylene glycol) (HO-PEG-OH, number-average molecular weight M_n=9.40×10^3, degree of polymerization DP=227, molecular weight distribution M_w/M_n=1.06, Aldrich), 2-(methacryloyloxy)ethyl phosphorylcholine (MPC, 96%, NOF Corp.), 4,4'-azobis(4-cyanopentanoic acid) (V-501, 98%, Wako), 2-(acrylamido)-2-methylpropanesulfonic acid (AMPS, 95%, Wako), 4-hydroxy-2,2,6,6-tetramethylpiperidinyl-1-oxy (HTEMPO, free radical, 98%, Aldrich), sodium hydrogen sulfite ($NaHSO_3$, Fluka, solution for synthesis, 38–40% in water), potassium persulfate ($K_2S_2O_8$, Aldrich, 99.99%), dimethylsulfoxide (DMSO, HPLC grade, POCH Gliwice), Griess reagent (1% w/v sulfanilic acid/0.1% w/v *N*-(1-naphtyl) ethylenediamine-dihydrochloride in 2.5% v/v H_3PO_4, Sigma), and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT, Sigma), trisodium citrate (≥99%), calcium sulfate (≥99.99%) (Sigma-Aldrich, Germany), activated partial thromboplastin time (aPTT) and prothrombin time (PT) reagents (Bio-Ksel, Poland), anti-factor Xa assay kits (Sekisui Diagnostics, USA), pentobarbital (Biovet, Poland), collagen (Chrono-log, USA), diagnostic kit for determination of calcium concentration (Cormay, Poland) were used as received. Water was purified using a Millipore Milli-Q system.

Methods

The block copolymers used in the studies were synthesized using reversible addition-fragmentation chain-transfer polymerization (RAFT).

Results and Discussion

Block copolymers containing PAMPS as the anionic block and PEG or poly(2-(methacryloyloxy)ethyl phosphorylcholine) (PMPC) as the neutral or zwitterionic block, respectively, were synthesized with various block length. It was found that the copolymers increased activated partial thromboplastin time (aPTT) (FIG. 1), prothrombin time and showed significant anti-fXa activity.

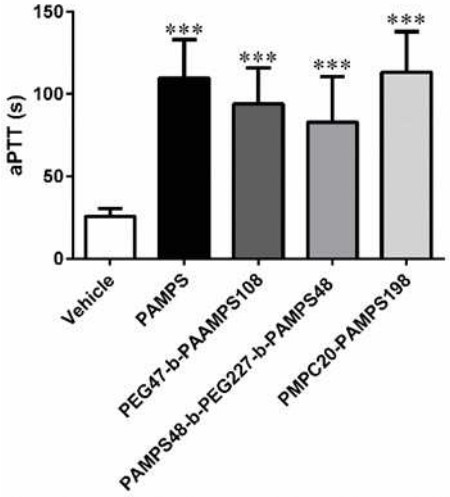

FIG. 1. Effects of PAMPS-based polymers on activated partial thromboplastin time (aPTT) in rats. ***p<0.01 vs. vehicle, unpaired Student's t-test. Results are shown as mean ± SD, n = 8-10.

Importantly, the polymers inhibited also platelet aggregation *in vitro*. However, in *in vivo* experiment the polymers inhibited, did not change or increased platelet aggregation. For PEG-PAMPS copolymers there was no change in the cardiorespiratory parameters, while for PAMPS homopolymer and PMPC-PAMPS copolymer with long PAMPS block a short term cardiac arrest and a significant decrease in the respiratory rate (RR) was found. All polymers significantly increased WBC in rats 30 minutes after intravenous administration. All the studied polymers showed anti-inflammatory properties.

Conclusions

The conclusions have to be based on the facts in evidence and should be limited to minimal speculation about the significance of the work.

Acknowledgments

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References

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