Clot lysis on lysine-coated peripheral stents preadsorbed with tPA

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INTRODUCTION

Thrombosis often occurs when an implanted medical device comes in contact with blood and frequently results in device failure. Anti-thrombotic strategies typically attempt to limit the deposition of coagulation proteins and the cellular components of blood, thus inhibiting thrombus growth. Our focus has been the development of a coating reagent containing pendant lysine groups that can be easily applied as a thin-film and covalently attached to a surface. We have shown previously that this reagent preferentially binds plasminogen and tissue-type plasminogen activator (tPA) from plasma[1], thereby promoting clot lysis[2]. In the work reported here, the lysine-coating reagent has been applied to a peripheral vascular stent and data on blood interactions of the coated stent have been developed.

MATERIALS AND METHODS

Proprietary peripheral stent "platforms" (herein referred to simply as "stents") were first treated with a polyvinyl pyrrolidone (PVP) priming agent and then coated with a polyacrylamide (PA) reagent containing covalently bound lysine residues. Similar procedures have been described previously[1]. Controls included uncoated stents, stents coated with PVP, stents with an outer coating of PA (no lysine) and stents with an outer coating of PA containing lysine with the α–amino groups free (α–lysine). The active stents had an outer coating of PA containing lysine with the ε–amino groups free (ε–lysine).

The clot lysing potential of the coated and uncoated stents was evaluated using a plasma clotting assay described previously[2]. Stents were placed in microtitre plate wells containing pooled citrated normal human plasma (PNP) for 3h and then rinsed extensively with tris-buffered saline (TBS). Stents were then "preadsorbed" with tPA in wells containing a 0.1 mg/mL solution of tPA for 30 min. Five consecutive 5 min rinses in TBS were then performed prior to placing the stents into wells containing 0.1 mL of fresh PNP and 0.1 mL of a 0.025 M $CaCl_2$ solution. The optical density at 405 nm was then measured at 10 sec intervals for a period of 1h at 37°C, with an increase corresponding to clot formation and a decrease to clot dissolution.

RESULTS AND DISCUSSION

As shown in Fig.1, the first appearance of a clot began at about 2 min following plasma recalcification for the PNP itself and for all five of the stent-plasma systems tested. The optical density reached a maximum value at 3.5 min for all systems. As indicated by the plateaus in the OD versus time curves, fully formed, stable clots developed in the wells containing PNP, the uncoated stent, and the PVP, PA and α–lysine (α–Lys) stents after roughly 4 min. In contrast, the maximum OD value in the well containing the ε–lysine (ε–Lys) stent was less than for the controls, and quickly returned to baseline after only 8 min indicating complete clot dissolution. Analogous experiments were conducted using cuvettes, and photographs were taken at ~5 min after clot formation was complete (Fig.2). As can be seen, the plasma remained clotted in the control cuvettes, whereas the cuvette containing the ε–lysine coated stent was completely clear.

From the data reported here, it is clear that clots formed on the ε–lysine coated stent were rapidly dissolved, thus providing an indication of the presence of active plasmin on the surface of the stent. These ε–lysine coatings may thus offer an alternative approach to the avoidance of thrombotic complications associated with these devices.

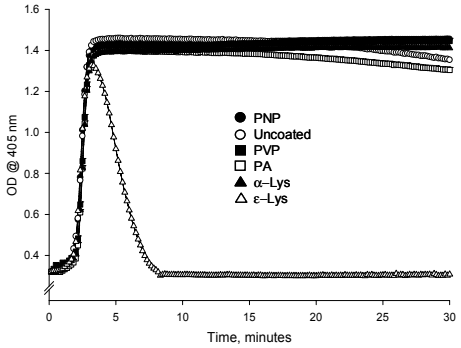

Figure 1: Clot dissolution by an ε–Lysine coated stent with preadsorbed tPA as measured in a plasma recalcification assay. Error bars are smaller than symbols. N=3.

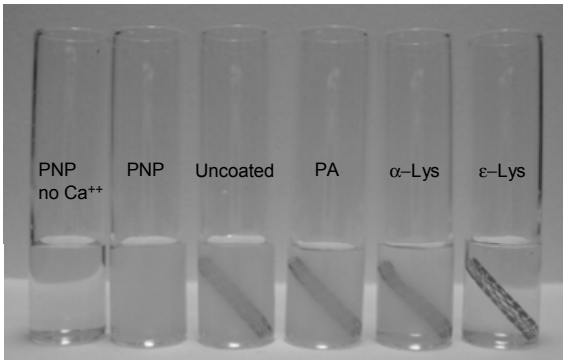

Figure 2: Clot formation in plasma containing stents preadsorbed with tPA. Photo taken approximately 5 min post clot formation.

REFERENCES

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