

SCA 4

TRUNCATED FORMS OF TISSUE FACTOR PATHWAY INHIBITOR CONTRIBUTE TO POST-CPB COAGULO-PATHY

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Introduction: Heparin attenuates tissue factor-dependent coagulation in part by releasing tissue factor pathway inhibitor (TFPI) from endothelium (1-4). Our group studied the effect of CPB on different forms of TFPI by using ELISA assays which specifically measure both the full-length form and those forms missing Kunitz-3 and the C-terminus. We observed that full-length TFPI increases during heparinization and returns to near-zero levels after protamine, while TFPI fragments lacking the C-terminus remain following protamine, and exhibit TFPI activity.

Methods: Following IRB approval and informed consent, we enrolled 12 adult cardiac surgery patients, and sampled plasma at 5 time points: prior to incision, 5 min after initiation of CPB, 1 hour on CPB, 10 min after protamine, and 1 day after surgery. The ELISA technique (American Diagnostica, Greenwich, CT) used a monoclonal antibody to Kunitz-1 of TFPI as the capture antibody, with a polyclonal antibody against Kunitz-1 or Kunitz-3 as detection antibody for assay of total and full-length TFPI, respectively. TFPI activity was assayed by the ability of test plasma to inhibit formation of a factor Xa chromogenic substrate.

Results: Figure 1 shows changes in total TFPI and full-length TFPI during cardiac surgery. Following reversal of heparin, full-length TFPI returned to levels almost lower than baseline, while total TFPI levels remained elevated ($p < 0.001$ by paired t-test). Assay of TFPI activity at these time points revealed that these truncated forms still carried TFPI activity (Figure 2, $p = 0.03$ by paired t-test and Wilcoxon signed rank test).

Discussion: Deficiency or proteolysis of TFPI are intriguing phenomena in several clinical settings (1, 2, 5, 6). Here, we show that TFPI forms lacking the C-terminus, and incapable of reattaching to endothelium following heparin reversal, remain in circulation following CPB and express TFPI activity. This may represent a novel cause of post-CPB coagulopathy.

References:

1. P. M. Sandset, B. Bendz, *Thromb Haemost* 78, 467 (1997).
2. L. B. Sun et al., *Surg Today* 30, 122 (2000).
3. G. J. Broze, Jr., G. W. Lange, et al. *Blood Coagul Fibrinolysis* 5, 551 (1994).
4. G. J. Broze, Jr., *Annu Rev Med* 46, 103 (1995).
5. A. A. Belaaouaj, A. Li, T. C. Wun, H. G. Welgus, S. D. Shapiro, *J Biol Chem* 275, 27123 (2000).
6. S. N. Doshi, J. D. Marmor, *Crit Care Med* 30, S241 (2002).

