072 **The effect of heparin on vascular endothelial function by monitoring intracellular accumulation of cGMP**

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Introduction: Endothelial dysfunction has been proposed as an important element in the low cardiac output state that may complicate the postoperative period following cardiac surgery. The endothelial L-arginine-NO-cGMP pathway is one of the critical determinants of vascular regulation [1]. We hypothesized that heparin, routinely used during cardiac surgery, could influence this pathway [2]. Our group tested this hypothesis by monitoring intracellular accumulation of cGMP in heparin-treated saphenous vein.

Method: Following IRB approval, saphenous vein was obtained from patients undergoing routine CABG surgery, prior to systemic heparinisation. Veins were dissected clean, cut into rings (4 mm width). Rings were then incubated in the absence of (control) or in the presence of unfractionated porcine heparin (5 IU/ml x 4 hours). Ongoing NO production and associated cGMP formation was assessed from the influence of the NO synthase inhibitor L-NAME (10 M^{-4} x 30 minutes). During the final 20 minutes of incubation a phosphodiesterase inhibitor (3-isobutyl-1-methylxanthine, 1 mM) was added to prevent cGMP breakdown. Intracellular cGMP was extracted by HCl (200 μl, 0.1M, 60 minutes) and quantified by radioimmunassay using murine polyclonal cGMP antibody. Experiments (n = 10) were performed in triplicate and results corrected for wet tissue weight.

Results: Mean absolute cGMP levels for control, heparin and L-NAME treated rings were 78.8 ± 24.3, 53.3 ± 13.5 and 45.0 ± 9.7 pmol per gram-wet tissue, respectively. This reduction in cGMP accumulation (77.6 ± 11.8 % and 42.6 ± 9.3 of control values, respectively) was significant (P<0.05) for both heparin and L-NAME treated rings. The reduction in cGMP accumulation to heparin was due to a significant reduction in 8/10 vessels when compared to control. Simultaneous administration of protamine sulphate completely eliminated this heparin response.

Discussion: The effect of L-NAME on cGMP levels suggests ongoing NO production contributes significantly to cGMP accumulation in human saphenous vein. This is the first observation that heparin exerts a negative influence on intracellular cGMP accumulation. This could be related to inhibition of ongoing NO production and stimulation of soluble guanylate cyclase, which requires further investigation. This observation might have important implications regarding the pathogenesis of endothelial dysfunction, especially in patients with already compromised function associated with a wide variety of disease states that receive heparin therapy.

References:

1 Moncada S, Palmer RM, Higgs EA. Nitric oxide: physiology, pathophysiology and pharmacology. *Pharmacol Rev* 1991; **43**: 109-142.

2 Upchurch GR Jr, Welch GN, Freedman JE, et al. High-dose heparin decreases nitric oxide production by cultured bovine endothelial cells. *Circulation* 1997; **95**: 2115-2121.