INTERACTIVE EFFECTS OF HOMOCYSTEINE AND COPPER ON ANGIOGENESIS IN PORCINE ISOLATED SAPHENOUS VEIN

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Following coronary artery bypass graft surgery (CABG) with saphenous vein there is a marked and protracted elevation of plasma homocysteine (Hcy) and copper (Cu), both of which are risk factors for cardiovascular disease (Jeremy et al., 2002; Shukla et al., 2006). Although the pathological consequences of this event are unknown, Hcy and Cu are known to interact to elicit endothelial dysfunction. It has been suggested that angiogenesis and the formation of a “neovasa vasorum” is an important adaptive event that mediates oxygenation of vein grafts and that impairment of this adaptation may compromise graft patency. Since angiogenesis involves the replication and migration of endothelial cells, a novel in vitro model of angiogenesis in isolated pig saphenous veins was developed and the effect of Hcy and Cu, alone and in combination on angiogenesis studied.

Saphenous veins from White Landrace pig were cut into 2 mm rings and embedded in a fibrin (formed by adding fibrinogen solution to thrombin solution) in 12-well plastic culture plates, incubated for two weeks and tubules counted daily. The effect of Hcy and CuCl$_2$, alone and in combination, penicillamine (a copper chelator) and modulators of superoxide formation on tubule growth then studied. Data are expressed as mean ± s.e.mean; n = 6. One-way ANOVA was used for statistical analysis. All studies were carried out in accordance with the Animal (Scientific Procedures) Act, 1986.

Tubule growth in cultured saphenous veins embedded in fibrin occurred in a time-dependent manner over 14 days, which was was inhibited by 1 µg / ml angiotatin and 10 nM thapsigargin. When measured at day 7, CuCl$_2$ alone at 1µM and 10 µM significantly augmented microtubule count (170 ± 15 and 210 ± 20, respectively) compared to control (140 ± 14) whereas at up to 1mM, Hcy alone had no effect. By contrast, Hcy and copper together markedly inhibited microtubule formation (count at 7 days at 1µM Cu and 10 µM Hcy; 85 ± 15 and at 10 µM Cu and 100 µM Hcy; 28 ± 6). The copper chelator, penicillamine, also inhibited tubule formation in a dose-dependent manner (IC$_{50}$; 2.5 µM). Significant inhibition of tubule formation and superoxide formation was elicited with 10 µM apocynin (NADPH oxidase inhibitor), 100 µM rotenone (an inhibitor of mitochondrial respiration) and 100 µM allopurinol (an inhibitor of xanthine oxidase), indicating that superoxide promotes, rather than inhibits, angiogenesis.

This novel in vitro method constitutes a reliable model for the study of angiogenesis in the pig saphenous vein. These data indicate that the increased and sustained increases in plasma Hcy and copper in patients undergoing CABG may exert a deleterious effect on graft patency by preventing the formation of a neo vasa vasorum, thereby promoting hypoxia. This effect was independent of superoxide and may be due to the formation of an inhibitory Hcy-Cu complex with anti-angiogenic properties.
