Darbepoetin does not protect against immediate ischaemia-reperfusion injury but enhances subsequent endothelium-dependent vasomotor function in patients with stable coronary artery disease

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Background Erythropoietin has actions on isolated endothelial cells to stimulate nitric oxide (NO) release and in vivo to increase circulating potentially reparative "endothelial progenitor" cells (EPC); it may also protect endothelial cells from damage due to ischaemia-reperfusion.

Aim The aim of the present study was to determine if the long acting erythropoietin analogue, darbepoetin improves flow mediated dilatation (FMD), a measure of endothelium-derived NO (EDNO) and protects against ischaemia-reperfusion.

Methods 34 patients (3 women, 40-75 years) with coronary artery disease (at least 50% angiographic coronary stenosis of one or more coronary arteries) were randomized to receive a single dose of darbepoetin 300 µg or saline placebo. Immunoreactive erythropoietin was measured by an enzyme linked immunospecific assay (ELISA). FMD was measured at the brachial artery using high resolution ultrasound and a 5 minute distal cuff occlusion to induce hyperaemic flow. Circulating EPC characterised according to surface markers (CD34+/VEGFR2+/CD133+) were enumerated by flow cytometry. Measurements were made immediately before darbepoetin/placebo and at 24h, 72h and 7 days after darbepoetin/placebo. At 24h FMD was repeated after 20 minutes ischaemia-reperfusion of the upper limb. A further group of 11 patients were studied according to the same protocol, all receiving darbepoetin, with omission of forearm ischaemia-reperfusion at 24h. The protocol was approved by the local research ethics committee and all patients gave written informed consent.

Results Immunoreactive erythropoietin peaked at 24h (660.2±25.1 vs 15.4±3.5 units in darbepoetin and placebo groups respectively, P<0.001) and remained elevated at approximately 5 fold baseline at 72h. There were no significant differences in haematocrit nor in circulating EPC between the placebo and darbepoetin group. FMD did not differ significantly between groups at 24h (before ischaemia-reperfusion) and immediate blunting of FMD after ischaemia-reperfusion at 24h was unchanged. However, at 72h, FMD increased from baseline in the darbepoetin group but not in the placebo group so that FMD (and change in FMD from baseline) was significantly greater in the darbepoetin group (change from baseline 1.7±0.29% and -0.41±0.44 in darbepoetin and placebo groups respectively, P<0.001). The increase in FMD at 72h after darbepoetin and ischaemia-reperfusion at 24h was significantly greater than that without preceding ischaemia-reperfusion.

Conclusion Darbepoetin does not protect against immediate ischaemia-reperfusion injury but enhances subsequent endothelium-dependent vasomotor function. This effect is unlikely to be mediated by EPC; a direct effect of darbepoetin on endothelial NO synthase to enhance EDNO mediated endothelial function may be stimulated by ischaemia-reperfusion.