Vasculoprotective Effects of Erythropoietin: New Developments and New Alternatives

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The body of evidence demonstrating the vasculoprotective effects of direct cell-based therapies to inhibit the acute response to vascular injury continues to grow. Delivery of autologous circulation-derived cells capable of assuming an endothelial phenotype to an injured arterial segment results in early reendothelialization and normalization of vascular function. Current applications of this approach require cell isolation and modification in vitro, followed by direct delivery. However, novel approaches are being developed to avoid the inherent translational concerns of this strategy. Broadly, these new approaches include the mobilization and targeting of cell populations capable of augmenting reendothelialization of injured arteries while limiting neointimal formation. Multiple cytokines have been shown to mobilize cells capable of assuming an endothelial phenotype (broadly known as EPCs). These cytokines include VEGF, G-CSF, GM-CSF, and erythropoietin (Epo). The article by Urao et al in this issue of Circulation Research extends the observation that Epo can mobilize EPCs while demonstrating its ability to enhance reendothelialization and inhibit neointimal formation after vascular injury in an NO-dependent manner. Taken together, these findings extend our understanding of the role of Epo as a tissue protectant and apply it to the setting of acute vascular injury.

The article by Urao tested the hypothesis that systemic delivery of recombinant human Epo would inhibit neointimal formation in a mouse carotid artery wire injury model. Epo (1000 IU/kg of body weight) was administered for 3 days beginning at the time of arterial injury. Morphometric analysis of serial arterial sections performed at 14 days after injury showed a 52% decrease in the neointimal area in the Epo-treated mice compared with the saline injected group. They further demonstrated that Epo-mediated inhibition of the neointimal formation is eNOS/NO-dependent in a series of well-constructed experiments treating wild-type mice with the nitric oxide donor, L-Arginine, the eNOS inhibitor, L-NAME, and performing arterial injury in eNOS-null mice.

Reendothelialization was greater in the Epo-treated animals than in the nontreated group as evidenced by staining of arterial tissue with Evans blue dye which was administered premortem. Immunostaining with antibodies to CD31 indicated that 55% of Epo-mediated reendothelialized area was derived from marrow-derived cells. Even though hemoglobin values were increased in response to Epo, there was no increase in thrombosis formation in Epo-treated animals compared with nontreated animals.

How does Epo produce a decrease in neointima formation and enhance reendothelialization? Urao and colleagues collected peripheral blood from Epo- and saline-treated animals 3 days after arterial injury. FACS analysis revealed a higher quantity of EPCs as determined by CD45dim/Fk-1 labeling in the Epo-treated group as opposed to the saline-treated group. To identify these cells as those of endothelial lineage, they were further cultured for 7 days in vitro. The majority of these CD45dim/FLK-1 cells bound FITC-conjugated BS-1 lectin and incorporated Dil-labeled acLDL. Again the relative numbers of endothelial like cells were greater in the Epo-treated mice than in the saline-injected controls. Of particular note, Epo receptor (EpoR) expression was localized on the regenerated endothelium especially on the CD31 endothelial cells in Epo-treated arteries compared with saline injected arteries.

Epo has been shown to have cytoprotective actions in tissue ischemia but may require doses higher than those to treat anemia. In the current article by Urao, human CD133 cells were used as surrogates for murine EPCs and it was shown that Epo markedly induced Akt-eNOS phosphorylation in these cells (which express Epo receptors). This effect may contribute to the protective effects of Epo on the neointimal hyperplasia. In spite of species differences, these studies provide a basis for understanding the murine vascular effects. One of the major points in this article is whether the Epo effects in the injured artery are in fact attributable to increased mobilization of EPCs from the bone marrow, as opposed to Epo-induced nonspecific increase in circulating NO pool attributable to induction of EpoR and eNOS in remote endothelial cells. The authors point out that the plasma NOx concentrations at various stages after injury of the Epo-treated mice were similar to those of the control mice. These important findings suggest that Epo-mediated action unlikely results from the nonspecific increase in circulating NO pool.

So is systemic delivery of Epo the future of vasculoprotection? Clinical experience with recombinant human Epo suggest that doses higher than 300 IU/kg (a dose resulting in maximal hematopoietic effects) may be related to an increase in adverse events in clinical studies. Notably,
A schematic demonstrating a proposed mechanism for dissecting the therapeutic effects of Epo according to receptor subtypes.

decreased survival was noted in anemic subjects treated with Epo with breast or head and neck malignancies. Specifically, procoagulant effects have been suggested in clinical studies\(^2\) and confirmed in in vitro cellular studies. Epo administration has been shown to increase circulating levels of thrombomodulin, von Willebrand factor, and a number of vascular adhesion molecules.\(^{10–12}\) Other cardiovascular effects of Epo include an increase in systemic blood pressure in subjects with renal failure as well as normals.\(^{13–15}\) Although procoagulant effects or other adverse cardiovascular effects were not noted in the study by Urao, the studies were performed in mice without existing cardiovascular pathology or substrate.

Given that Epo has distinct hematologic effects and is associated (at the higher doses used in the study by Urao) with adverse events in clinical studies, are there alternatives that might provide the benefit without the risks? The article by Urao and a recent publication in Proceedings of the National Academy of Sciences by Coleman and colleagues\(^6\) suggest that alternatives might exist to provide the vasculoprotective effect without attendant risks. In the article by Urao, \(L\)-arginine administration (2.25% in drinking water 7 days before injury and 14 days after injury) significantly reduced intimal formation in this murine model. As \(L\)-arginine supplementation is well tolerated in general and has been used in patients with vascular disease,\(^17\) it may be a reasonable alternative to Epo use as a vasculoprotectant. However, two features of this comparison remain uncertain. First, in the article by Urao the effects of \(L\)-arginine on EPC mobilization and reendothelialization were not demonstrated, so the mechanism of its effects in this model has yet to be defined. Second, at least one human clinical trial of \(L\)-arginine supplementation failed to inhibit neointimal formation after coronary stenting.\(^18\) In a small placebo-controlled randomized study \(L\)-arginine infusion during and after the procedure followed by oral administration (6 g/d) failed to limit intimal formation in spite of enhanced arginine levels. Obviously, dosing issues must be considered when comparing these studies as well as the inherent challenges of clinical translation.

A recent study highlights the rapid development of compounds that lack the erythropoietic features of Epo but maintain the tissue protective features.\(^{16}\) In this article, the development of carbamylated Epo (CEPO) which has no affinity for the homodimeric Epo receptor (EPOR) but uses an alternative heteromeric receptor complex is described (Figure). It is the activation of this alternative complex that is thought to regulate the tissue protective effects of Epo whereas activation of the homodimeric complex regulates erythropoiesis.\(^{19}\) CEPO was shown to stimulate EPC accumulation in the bone marrow of mice without erythropoietic effects or hypertensive effects seen with Epo. Although it has not been shown that CEPO can affect neointimal formation as demonstrated in the article by Urao, the potential for nonerythropoietic vasculoprotectants is significant.

As in any therapeutic revolution, the hyperbolic expectations of cardiovascular cell therapies are likely to be succeeded by practical yet important realities. In the race to define (and perhaps own) the cells of choice, it is possible that systemic delivery of peptides or small molecule mediators of cell mobilization and targeting may ultimately hold the most promise. This promise is an extension of basic science research, the basic science necessary for true innovation.

Acknowledgments

This work was supported by National Institutes of Health grant HL75566. We thank Megan Crouch and Cheri Mueske for their technical support.

References


**Key Words:** cytokines ■ cell therapy ■ vascular injury
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Circ Res. 2006;98:1341-1343
doi: 10.1161/01.RES.0000228463.96905.45
Circulation Research is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7330. Online ISSN: 1524-4571

The online version of this article, along with updated information and services, is located on the
World Wide Web at:
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