Introduction

- Injury of the vascular endothelium represents a critical feature in the early stages of vascular disease.\(^1\)\(^-\)\(^3\).
- Erythropoietin (EPO) is a cytokine which is induced by hypoxia and promotes erythropoiesis (red blood cell formation), via ligation to a homo-dimeric EPO receptor (EPOR).\(^4\)\(^-\)\(^5\).
- In recent years it became clear that EPO is expressed in several tissues and has multiple tissue-protective and reparative activities mediated by a distinct heterodimeric receptor (EPOR and a common beta-subunit).\(^6\)\(^-\)\(^7\)
- A new generation of EPO derivatives that are tissue protective but not erythropoietic have therefore been developed. These compounds bind to the EPOR-\(\beta\)CR complex but not the EPOR homo-dimer.\(^8\).
- EPO and its non-erythropoietic derivatives have been studied in models of cardiovascular injury and have been reported to promote wound healing in the skin.\(^2\).

Aim and Hypothesis

**Aim:** Investigate the tissue protective effect of EPO and its analogues in an *in vitro* model of wound healing under hypoxia and normoxia.

**Hypothesis:**
1. EPO and its analogue exert similar tissue protective effects
2. Oxygen level has an effect on the activity of EPO and its analogues

Methods and Results

1. **In vitro wound healing model**
   - **Wound healing model:** Scratch assay
   - **Cells:** Bovine aortic endothelial cells (BAECs)
   - **Oxygen level:** Normoxia (21% oxygen) and hypoxia (5% oxygen)
   - **EPO analogues tested:** CEPO, pHBSP, Scr-pHBSP

2. **Proliferation assay**
   - **(Trypan blue viability method)**
   - a. Seed cells at 1 x 10\(^5\) cells/mL in 96 well plate
   - b. Add EPO or EPO analogue at concentrations (0-10 ng/mL)
   - c. After 0, 24 and 48 h add trypan blue and count viable cells

3. **Migration assay**
   - **(Using Boyden chamber)**
   - Incubate for 4 h
   - Fix and stain filter paper with Diff Quick Stain
   - Microscope (40x)

4. **Expression of EPOR and \(\beta\)CR**
   - a. **Gene expression** (Real time qPCR)
     - Difference in expression of EPOR and \(\beta\)CR gene were quantified and compared under normoxia and hypoxia using \(\Delta\Delta\)Ct method
   - b. **Protein expression**
     - Western blot
     - Immuno fluorescence
     - In both methods EPOR and \(\beta\)CR expression on the protein level were compared under normoxic and hypoxic conditions using antibodies specific for each protein

Conclusion and Clinical significance

**Conclusion:**
- Non erythropoietic analogues of EPO showed similar reparative effect to EPO.
- The effects of EPO and its analogues were enhanced by hypoxia and probably mediated by effects on cell migration and proliferation.

**Clinical significance:**
- Non erythropoietic EPO derivatives may represent a potentially safer and more effective intervention for the treatment of cardiovascular disease as atherosclerosis.
- Expression of EPOR is increased under hypoxia while expression of \(\beta\)CR is not affected.
- This requires further investigation in *in vivo* models of vascular injury, including atherogenesis and vascular re-stenosis.

References