Reviewer’s report

Title: Local Erythropoietin and Endothelial Progenitor Cells Improve Regional Cardiac Function in Acute Myocardial Infarction

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Reviewer: Peter Van der Meer

Reviewer’s report:

EPO influences a broad array of cellular processes that include protection against ischemic injury and angiogenesis, possibly through stimulation of endothelial progenitor cells and in situ proliferation of endothelial cells. In recent years, expression of functional EPO-receptor has been demonstrated in non-hematopoietic cells and organs including brain, kidney and cardiovascular tissue. These findings suggest that besides stimulating hematopoiesis, EPO may play a role as a pleiotropic survival and growth factor. Indeed, many experimental studies have shown protective effect of exogenous EPO treatment in different tissues, including the heart. In animal models, EPO administration during cardiac ischemia/reperfusion injury reduces the infarct size and improves hemodynamic parameters of the left ventricle. On the other hand also endothelial progenitor cells have shown beneficial effects in experimental myocardial infarction.

The study of Stein et al. combines both strategies. The authors show in vitro that EPO decreases apoptosis of EPCs. The in vivo part of the project shows the effect of local EPO injection alone, EPC injection in the border zone without EPO and the combination of EPO with EPC injection. The authors find that the combination gives the best contractility in the border zone as elegantly shown by MRI (regional wall motion). This is accompanied by increase vasculogenesis and survival of transplanted cells. However no beneficial effect on LV ejection fraction.

In general this is an interesting study, however I have several issues:

Major issues

Since MRI is a powerful imaging tool, more data should be presented. What were the effects on LV end diastolic diameter/volume and LV end systolic diameter/volume.

No baseline cardiac function measurements (MRI or echo) were performed directly after myocardial infarction (and injection). This would allow the authors to really substantiate the claim that the effects shown are indeed related to the injected cells with EPO.

The authors show nicely that capillary density increases with the combination of EPO and EPCs. Post myocardial infarction remodelling plays an important role. Did the intervention have an effect on the myocyte size? In addition the authors should also report the capillary to myocyte ratio in order to prove vasculogenesis.
The authors did not include a sham group, so no information is available to which extent the cardiac parameters improved (including LV function and effects on vasculogenesis).

Further information is needed on how many cells survived after 4 weeks. How many cells indeed incorporated into the capillaries, were there capillaries formed entirely from human C31+ cells? How reliable is double staining with TUNEL. Is it possible that cells undergoing apoptosis and labelled with TUNEL already lost the CD31 receptor which may lead to false negative findings. Please comment.

Minor issues:
- The authors should mention the number of animals used in the abstract
- The authors may consider to divide the MRI section in two parts. First they actually validate their methods to measure regional wall motion (total occlusion or Ischemia/Reperfusion injury. Then in the second part they report data on the actual treatment effect of EPO with/without EPCs. This is confusing.

**Level of interest:** An article of importance in its field

**Quality of written English:** Acceptable

**Statistical review:** No, the manuscript does not need to be seen by a statistician.