**Reviewer’s report**

**Title:** Epoetin beta pegol for treatment of anemia ameliorates deterioration of erythrocyte quality associated with chronic kidney disease

**Version:** 0  **Date:** 06 Jun 2017

**Reviewer:** Naoko Minegishi

**Reviewer’s report:**

The authors elucidated the effect of Epoetin beta pegol (C.E.R.A) on the quality of erythrocyte in the rat model of chronic kidney disease (CKD). They clearly showed the improvement of MCV, elongation index and life span of erythrocytes by C.E.R.A. treatment in CKD model rats ((UNX-Thy1 group), which had hemi-nephrectomy and ant-rat CD90 monoclonal antibody treatment. These findings will be clinically important to understand the long-term symptoms and prognosis of CKD patients under treatment with C.E.R.A. or other erythropoietin derivatives.

1. The authors showed the increase of intracellular calcium in the erythrocytes of UNX-Thy1-vehicle group, but not in UNX-Thy1-C.E.R.A group (Fig.4). From these findings, the authors claimed that an increase in MCV and a decrease in erythrocyte deformability in CKD patients might be induced by calcium loading in erythrocytes. However, the authors only demonstrated the observatory findings in this paper.

It is advisable to comment on some evidences that explain the mechanisms of calcium signaling on erythrocyte deformability, and the effect of C.E.R.A on these mechanisms.

2. The effect of C.E.R.A on the MCV, deformability and longevity of erythrocytes were only partial in UNX-Thy1-C.E.R.A group (Table 1, Fig.2, Fig.3), while the level of calcium concentration in erythrocyte and the hemoglobin concentration in UNX-Thy1-C.E.R.A group, were almost equal to the sham-operation group. It seems important to discriminate the erythropoietin-dependent and -independent mechanisms on the hematological symptoms in CKD model rats.

**Are the methods appropriate and well described?**
If not, please specify what is required in your comments to the authors.

Yes

**Does the work include the necessary controls?**
If not, please specify which controls are required in your comments to the authors.

Yes

**Are the conclusions drawn adequately supported by the data shown?**
If not, please explain in your comments to the authors.
Yes

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