Peer Review File

Article information: https://dx.doi.org/10.21037/atm-21-4357

Reviewer A

This meta-analysis included 10 related RCTs, including phase 2, phase 3 and phase 4 trials and is well organized. The results showed that ROX is effective in increasing Hb level, improving iron utilization parameters and is safe for DD-CKD patients with anemia. Compared to ESA, ROX performs better in improving several iron parameters. But the difference of their effect on Hb level requires further confirmation.

However, more than one systematic review on this topic has already been published. One of the articles written by Li Zhang et al. (DOI:[Zhang, 2021 #81]) has more comprehensive analysis although the studies included are less than this article. The results are also similar to previous articles, leading to the lack of innovation.

There are some details that need to be revised:

1. The baseline characteristics should be analyzed to make sure that the results are reliable.

Reply: Thank you so much for your suggestions. We added a more detailed qualitative analysis to the result section, and we also added a **supplementary S4 table** describing all added baseline characteristics of both ROX and control groups.

Changes in the text:

We added the following to the characteristics of included Studies

In the ROX group, the Hg ranged from 8.4 to 11 g/dl, TSAT% ranged from 27% to 43%, ferritin ranged from 190.2 to 1002.02 ng/ml, and finally, hepcidin ranged from 142.8 to 327.1 ng/mL. The baseline laboratory values of the included patient are summarized in **Supplementary Table S4**.

2. In the discussion, the author mentioned dosages and the time of initial dialysis may influence the results. So another subgroup analyses can be performed according to these two factors.

Reply: Thank you so much for your suggestion. Unfortunately, due to different doses and usage of adjustment doses bases on Hb levels make specifying doses difficult. Even if we rely on specific /mean/largest dose this makes the result unreliable. For example, some studies started with a low dose of ROX and then gradually increased the dose (Provenzano et al. 2016 and NCT02278341), and other RCTs did the ROX dosage according to the weight. (Chen et al. 2017 and Chen et al. 2019).

Also, the time of initial analysis is overlapping and most of the RCTs reported a range of the time of the dialysis initiation as not all the patients were started on the dialysis at the same time. For example, the time ranged from 2 to 5 weeks in Nct01888445, from 2 weeks to 4 months in Nct02174731, and ≥2 weeks and ≤4 months in Provenzano et al. 2021.

However, we tried to conduct subgroup analysis as shown below but undetailed information about doses and times forces us to not recommend adding them to the manuscript as they will mislead the readers. However, we attached the meta-analysis's results below, and we would be happy to consider it if you wish.

Changes in the text: None

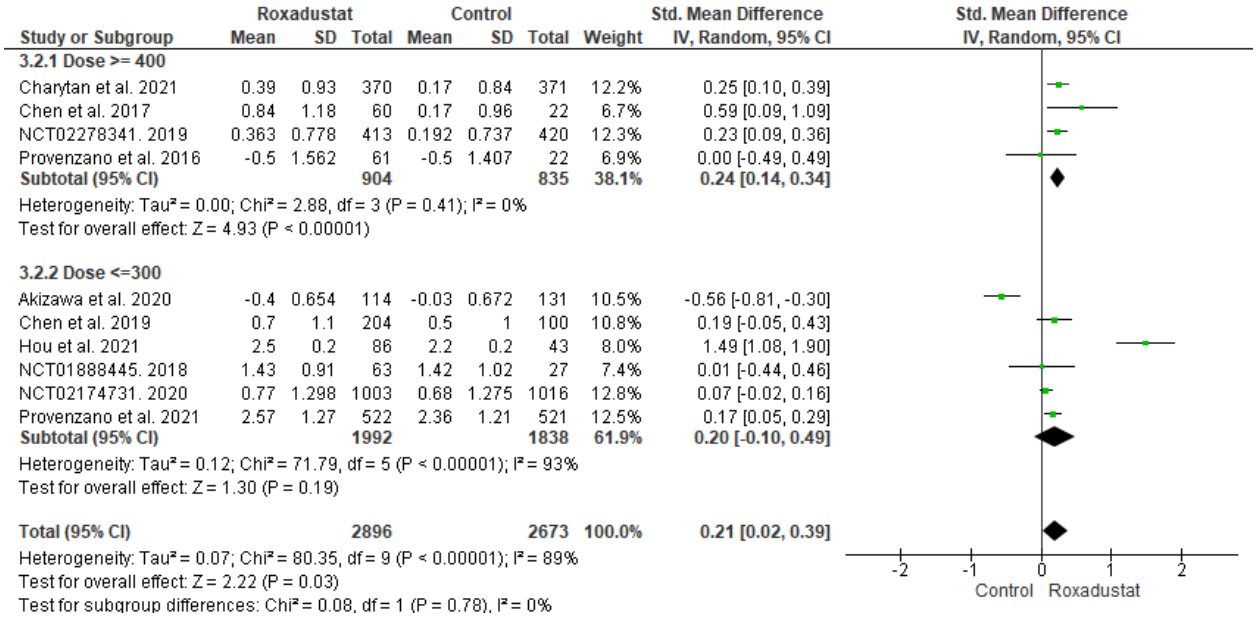

Figure 1 Forest plot of comparison: 3 Subgroup analysis according to the ROx dosage, outcome: 3.2 Hemoglobin.

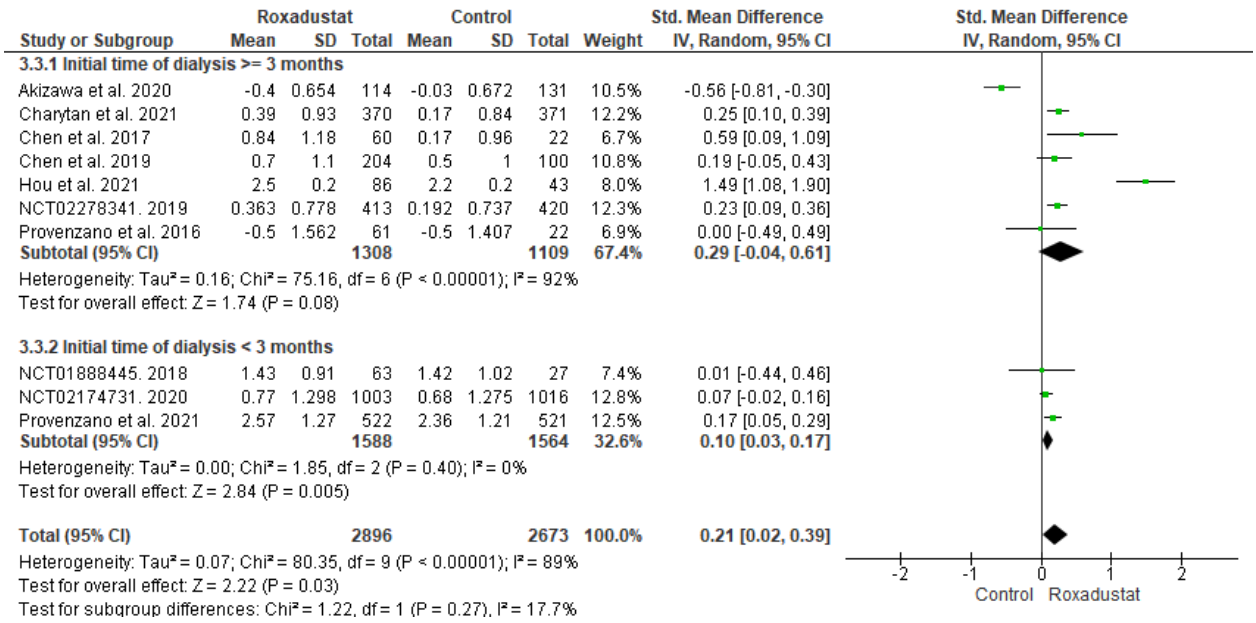

Figure 2 Forest plot of comparison: 3 Subgroup analysis according to the initial time of dialysis , outcome: 3.3 Hemoglobin.

3. What are the detailed side effects? The author can compare their difference between ROX and ESA in detail, which can provide instructions for the clinical doctors and improve the innovation of the article as well.

Reply: Thank you so much for your comment. We did a meta-analysis of the reported adverse effects between both groups, and we discussed in detail the difference between ROX and ESA.

Changes in the text:

All meta-analysis figures for the adverse effects were added.

We added the following in the result section:

ROX group showed more gastrointestinal adverse effects compared to the control group (RR: 1.40; 95% CI: 1.04, 1.88; P= 0.03) (Figure 4, Forest plot C). However, there was no difference between both groups regarding cardiovascular adverse effects; injury, poisoning, and procedural complications; muscle spasm; infection or infestation; upper respiratory tract infections; hypertension; and hyperkalemia (Figure S2-S8, Supplementary Material S2)

We added the following in the discussion.

However, in our meta-analysis, there was no difference between ROX and the control group in regard to hyperkalemia.

And our results confirmed that ROX group had higher gastrointestinal adverse effects compared to the control group

ESA therapy is associated with an increased risk of cardiovascular adverse events [Pfeffer, 2009 #82] [Singh, 2006 #83]. Our results showed that there was no significant difference between ROX and the control group in regard to cardiovascular adverse events. However, few of the included studies were not powered to detect the cardiovascular adverse effects like Akizawa et al [Akizawa, 2020 #48], and in anther RCTs the included patients with any cardiovascular events in past were excluded [Chen, 2017 #13]. However, Provenzano et al 2016, reported three deaths for patients who were having significant cardiovascular risks, but none of the deaths was attributed to ROX. Clearly, further research will be needed to validate the cardiovascular effects of ROX.

4. It would be better to add necessary units in the Figure 3. For example, Mean [g/dL].

Reply: Thank you so much for your reply. We added the units in Figure 3 as requested.

Changes in the text:

We added the footnote for the Figure 3

Hemoglobin (g/dl); hepcidin (ng/mL); TIBC(μmol/L); serum iron(μmol/L); transferrin(g/L); ferritin(ng/ml); TSAT(%)

df, degrees of freedom; I^2, I-squared; IV, inverse variance; CI, confidence interval.

5. The abbreviations “serious TEAEs” in Figure 4 is not consistent with “serious side effects” in the text.

Reply: Thank you so much for your note. We changed the abbreviations in Figure 3 to serious side effects.

Changes in the text:

Figure 4 was changed.

Reviewer B

The authors included 10 RCT studies. It demonstrates that ROX decreases hepcidin

levels and increases Hb levels.

1. In how many studies was CRP assessed?

Reply: Thank you so much for your comment. There were five RCTs assessed CRP and we added **Supplementary Material, Table S5**. summarized the patient included in each RCT according to CRP and we discussed those results in of the result

Change in the text:

We added the following to the result section, Characteristics of included Studies
Five RCTs reported the role of ROX in the inflammatory process, and they assessed the C-reactive protein (CRP) as a factor [Akizawa, 2020 #48;Charytan, 2021 #49;Chen, 2019 #50;Hou, 2021 #53;Provenzano, 2021 #52]. The patients were divided into two groups according to the upper limit of normal (ULN) for CRP. The ULN was 3 in [Akizawa, 2020 #48;Hou, 2021 #53] and 4.9 in [Chen, 2019 #50]. The CRP for most of the included patients was less than the ULN, and CRP was less in the ROX than the control group **Supplementary Material, Table S5**.

2. What about cardiovascular events across the 2 studies?

Replay: Thank you so much for your comments. Six RCTs reported cardiovascular adverse events, and we did a meta-analysis for the cardiovascular adverse events. Our result showed that there was no significant difference between both groups.

Change in the text:

We added the following in the results section:

ROX group showed more gastrointestinal adverse effects compared to the control group (RR: 1.40; 95% CI: 1.04, 1.88; P= 0.03) (Figure 4, Forest plot C). However, there was no difference between both groups in regard to **cardiovascular adverse effects**; injury, poisoning, and procedural complications; muscle spasm; infection or infestation; upper respiratory tract infections; hypertension; and hyperkalemia (Figure S2-S8, Supplementary Material S2)

We added the following in the discussion section.

ESA therapy is associated with an increased risk of cardiovascular adverse events [Pfeffer, 2009 #82] [Singh, 2006 #83]. Our results showed that there was no significant difference between ROX and the control group in regard to cardiovascular adverse events. However, few of the included studies were not powered to detect the cardiovascular adverse effects like Akizawa et al [Akizawa, 2020 #48], and in anther RCTs the included patients with any cardiovascular events in the past were excluded [Chen, 2017 #13]. However, Provenzano et al 2016, reported three deaths for patients who had significant cardiovascular risks, but none of the deaths was attributed to ROX. Clearly, further research will be needed to validate the cardiovascular effects of ROX.

3. .There were more TEAEs in the hepcidin group : is it possible to detail them ?

Reply: Thank you so much for your comment. We discussed the TEAEs in detail as requested.

Change in the text:

We added the following in the result section:

ROX group showed more gastrointestinal adverse effects compared to the control group (RR: 1.40; 95% CI: 1.04, 1.88; P= 0.03) (Figure 4, Forest plot C). However, there was no difference between both groups regarding cardiovascular adverse effects; injury, poisoning, and procedural complications; muscle spasm; infection or infestation; upper respiratory tract infections; hypertension; and hyperkalemia (Figure S2-S8, Supplementary Material S2)