

Oral treatment with PBI-1402 increases hemoglobin, hematocrit and red blood cell formation in 5/6-nephrectomized rats, a model mimicking human end-stage renal disease



PROMETIC

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ABSTRACT

Background: PBI-1402 is a novel orally active low molecular weight compound with erythropoiesis stimulating activity, via a mechanism of action distinct from erythropoietin (EPO).

Aim: To better define the impact of PBI-1402 on red blood cell formation, we studied the effect of PBI-1402, compared to EPO, in anemia associated with chronic renal failure induced by 5/6 nephrectomy (Nx).

Methods: Male Sprague Dawley rats (6 week-old) were subjected to Nx or sham surgery. Treatment started at day 21 with water, PBI-1402 (200 and 400 mg/kg, oral, once a day), or rhEPO (25 U/rat, subcutaneous injection, twice a week) and was ended at day 56. Rats were sacrificed on day 63. Anemia markers were followed along the treatment.

Results: As shown in **Table 1**, our CRF model induced a significant anemia. Administration of PBI-1402 at 200 mg/kg induced a significant increase in reticulocyte count, with no modifications in hemoglobin (Hb) level. However, PBI-1402 at 400 mg/kg was associated with a significant increase in Hb and hematocrit levels compared to value observed on day 21 (before drug dosage). On the other hand, administration of rhEPO completely corrects anemia.

Table 1 Effect of PBI-1402 and EPO on Nx rat anemia

Group	Dose	Reticulocytes % Day			Hemoglobin g/L Day			Hematocrit L/L Day		
		21	42	63	21	42	63	21	42	63
Sham		2.3	2.7	2.5	173	167	172	0.49	0.47	0.48
Nx		2.2	7.6*	5.7*	134	110	117	0.37	0.31	0.36
Nx + PBI-1402	200 mg/kg	1.9	8.7*	5.3*	133	111	129	0.37	0.31	0.36
	400 mg/kg	2.9	11.0*	5.9*	125	132	150*	0.35	0.38	0.42*
Nx + EPO	25 U	5.0	10.7*	2.6	135	165*	167*	0.37	0.46*	0.46*

*Significantly different from value on day 21 p<0.05

Conclusion: PBI-1402 partly corrects anemia in CRF even in the presence of insufficient EPO concentration. PBI-1402 induces red blood cell formation in a dose-dependent manner. These results suggest that PBI-1402 offers the potential for a novel therapy of anemia associated with chronic renal failure.

BACKGROUND

PBI-1402 increases the production of immature progenitor stem cells (CFU-GEMM) from bone marrow and promotes the maturation of BFU-E and CFU-E. These effects result in increased production of red blood cells (RBC) in three animal models: immunosuppressed (chemo- and radiotherapy) mice and Nx rats. Furthermore, in a phase I clinical trial, PBI-1402 increased significantly both the relative and absolute numbers of reticulocytes in healthy volunteers. A phase Ib/II clinical trial confirmed that PBI-1402 increased RBC and Hb levels in patients with chemotherapy-induced anemia (CIA).

METHODS

5/6-NEPHRECTOMIZED RAT MODEL

Male Sprague Dawley rats (6 week-old) were subjected to 5/6 nephrectomy or sham operations.

Experimental Design:

Day 0: Partial Nx (2/3 left kidney).

Day 7: Total Nx (right kidney). Follow-up for 14 days (BW measurement and food intake).

Day 21: Measure GFR (creatinine clearance). Serum creatinine must be >125 mmol/L to be enrolled. This is equivalent to ~500 in humans or stage 5 ESRD. Start treatment (PBI-1402; oral once a day, EPO; sc, 25 U diw).

Day 42: Measure GFR (creatinine clearance).

Day 56: Stop treatment.

Day 63: Sacrifice.



RESULTS

Effect of oral treatment of PBI-1402 on reticulocytes in 5/6-Nx rats.

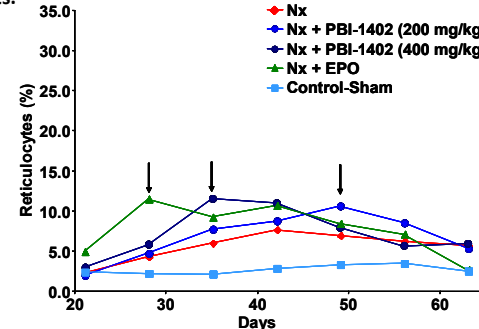


Figure 1

Effect of oral treatment of PBI-1402 on hemoglobin in 5/6-Nx rats.

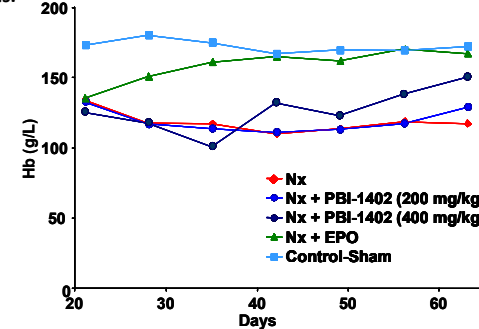


Figure 3

Effect of oral treatment of PBI-1402 on red blood cells in 5/6-Nx rats.

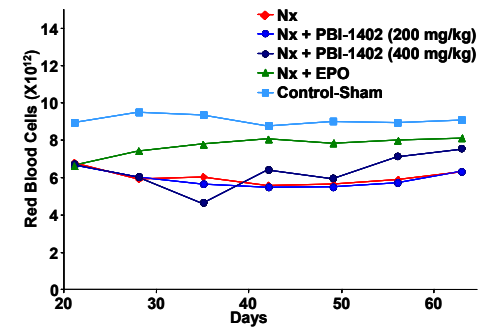


Figure 2

Effect of oral treatment of PBI-1402 on hematocrit in 5/6-(Nx) rats.

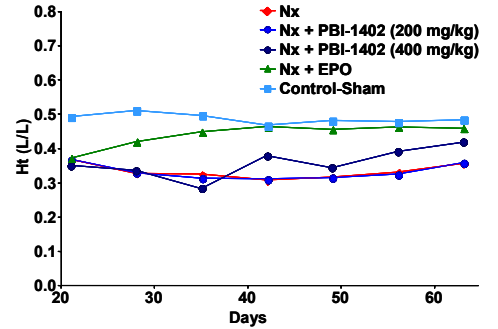


Figure 4

DISCUSSION

Figure 1 represents the effect of treatment of PBI-1402 on reticulocytes in Nx rats. Reticulocyte numbers in the control group (Sham) remained at a low level during the observation period. In the 5/6-Nx group, reticulocyte count increased steadily from 2% at day 21 to almost 8% at day 42. This increase in reticulocytes probably results from the anemia induced by the nephrectomy. However, this increase in reticulocytes is not sufficient to translate to red blood cell formation (**Figure 2**). Treatment of Nx rats with PBI-1402 induced a further increase in reticulocyte count. In fact, at 200 mg/kg of PBI-1402, reticulocytes increased up to 10.5% at day 49. At 400 mg/kg of PBI-1402, a huge increase in reticulocytes is observed (up to 11.5%). In addition, it should be noted that the maximum increase is attained faster (T_{max} : day 35) at the higher dose. The observed reduction in reticulocyte count at a high dose of PBI-1402 (day 42-63) corresponded with the increase in circulating red blood cells (**Figure 2**), hemoglobin level (**Figure 3**) and hematocrit (**Figure 4**) at day 42 and 49. Finally, rhEPO (25 U/rat, subcutaneous injection, twice a week) stimulated the formation of reticulocytes with a maximum observed between day 28 and day 42 resulting in a significant increase in hemoglobin (C_{max} : 32 g/L) at an earlier time (day 35). **The delay in reticulocyte expansion in PBI-1402-treated compared to EPO-treated Nx rats appears due to their effect on different hematopoietic progenitor targets, PBI-1402 acts on more immature cells (CFU-GEMM) than EPO (BFU-E and CFU-E).**

CONCLUSION

These results suggest that PBI-1402 can correct anemia in chronic kidney disease and even in the presence of insufficient endogenous EPO, PBI-1402 induces red blood cell formation in a dose-dependent manner. In a CRF model, treatment with PBI-1402 promotes:

- ❖ ↑ reticulocytes
- ❖ ↑ red blood cells
- ❖ ↑ hemoglobin
- ❖ ↑ hematocrit

These results also suggest that **PBI-1402 offers the potential for a novel therapy** of anemia associated with chronic renal failure.