

Nephroprotection: Oral treatment with PBI-1402 prevents doxorubicin-induced nephrotoxicity in mice

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PROMETIC

ABSTRACT

Background: PBI-1402 is a novel orally active low molecular weight compound which induced sufficient erythropoiesis to raise RBC and Hb in patients chemotherapy-induced anemia (CIA) via a mechanism of action distinct from erythropoietin (EPO). Clinical use of doxorubicin (Dox), a potent anticancer agent, is associated with marked nephrotoxicity characterized by tubulointerstitial lesions.

Aim: The aim of this study was to investigate the protective effect of PBI-1402 on Dox-induced nephrotoxicity in mice.

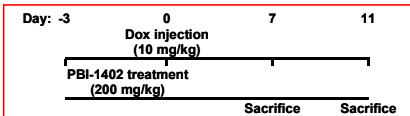
Method: Balb/c mice were randomized in three groups: Control, Dox and Dox + PBI-1402. Mice were prophylactically treated with oral administration of PBI-1402 (400 mg/kg) from day -3 to day 10. On day 0, mice were immunosuppressed with 10 mg/kg i.v.) Dox.

Results: Prophylactic treatment with oral administration of PBI-1402 significantly reduced Dox-induced nephrotoxicity. Furthermore, PBI-1402 prevented Dox-induced albuminuria. Histological lesions were also significantly ($p < 0.05$) reduced in PBI-1402-treated mice (Lesion scores determined by HPE staining at Day 7; Dox: 1.03; Dox + PBI-1402: 0.18; at Day 11; Dox: 2.0; Dox + PBI-1402: 0.72). Dox induces CTGF mRNA expression in the kidney. Prophylactic treatment with PBI-1402 induces a significant ($p = 0.03$) reduction of the expression of CTGF (33%) in the kidney.

Conclusion: These results suggest that PBI-1402 has protective effects against Dox-induced nephrotoxicity in the acute phase of toxicity and encourage further studies regarding its use as a potential nephroprotective agent. The protective effect of PBI-1402 relies, at least in part, on an antifibrosis effect as observed with decrease of CTGF expression.

METHODS

Dox-induced Nephrotoxicity: The effect of PBI-1402 was evaluated in Dox-immunosuppressed mice (Balb/c mice; 6-10 week old) a model used for acute renal failure. Histological changes were evaluated by quantitative measurement of glomerular, tubular and interstitial injuries in the cortex of the kidney.



Glomerular injury was assessed by grading protein deposition, mesangial and endothelial cell proliferation or sclerosis, epithelial cell (crescent) proliferation. Interstitial injury was assessed by grading inflammation and fibrosis (collagen formation). The lesions were graded on a scale from 0 to 3: 0=normal; 0 to 1=0-25%; 1 to 2=25-75% and 2 to 3=>75% damaged tissue. The tubular and interstitial injury was averaged and this resulting score was added to the glomerulus injury score for a total grading scale of 6.

RESULTS

Figure 1 represents the effect of prophylactic treatment of PBI-1402 on serum albumin in murine Dox-induced nephrotoxicity. Untreated Dox-induced nephrotoxicity group (Dox) exhibits a significant reduction in serum albumin concentration from day 4 to day 11 compared to control group (no Dox). Oral treatment with PBI-1402 (Dox + PBI-1402 group) prevents Dox-induced albuminuria.

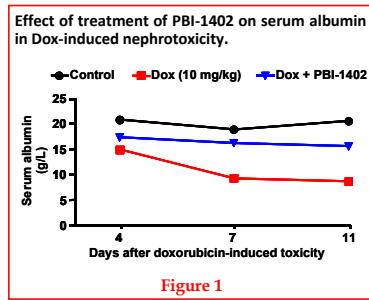


Figure 1

Figure 2 represents the effect of PBI-1402 on serum erythropoietin (EPO) concentration. Dox also induces anemia as demonstrated by an increase in serum EPO concentration compared to control animal (no Dox). Prophylactic treatment of mice with PBI-1402 protects against Dox-induced anemia as demonstrated by a lower production of EPO.

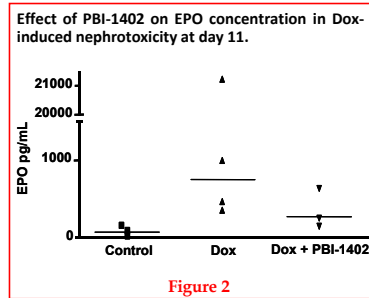


Figure 2

Figure 3 is a representative renal cortical sections in Dox-vehicle and Dox-PBI-1402 treated mice after the induction of Dox-induced nephrosis. Mice with Dox-induced nephrosis exhibited severe lesions in both glomerular and tubular compartments. Dox induces glomerular and tubulointerstitial injuries and necrosis. Increased interstitial volume due to protein deposition is predominantly observed. These lesions were mostly abrogated by treatment with PBI-1402.

Histological micrographs of renal tissues from vehicle and PBI-1402-treated Dox-induced nephrotoxicity in mice at day 11. Hematoxylin-Eosin staining (x40, x100, x400).

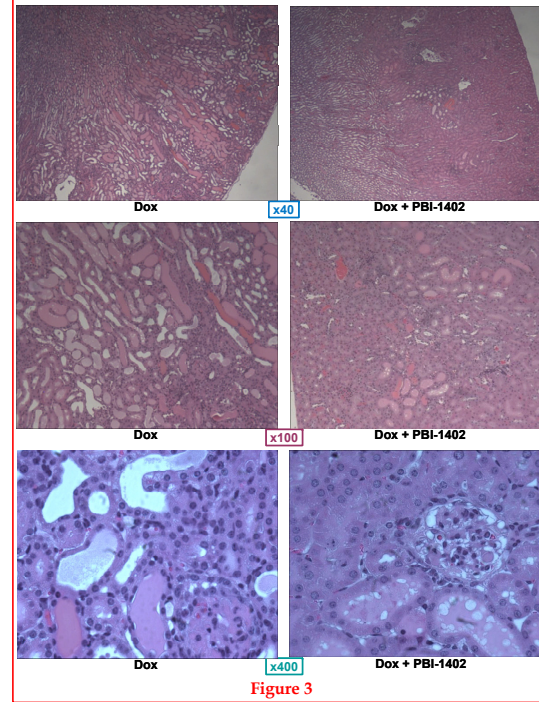


Figure 3

Figure 4 represents the effect of PBI-1402 on histological lesions induced by Dox. Examination of renal tissue 7 and 11 days after Dox induction demonstrates severe tubular damage in the vehicle-treated animals with multiple tubules containing obstructing protein. Histological lesions were significantly ($p < 0.05$) reduced in PBI-1402-treated mice (lesion scores determined by HPE staining at Day 7 Dox: 1.03; Dox + PBI-1402: 0.18; at Day 11; Dox: 2.0; Dox + PBI-1402: 0.72).

Effect of PBI-1402 on histological lesions in Dox-induced nephrotoxicity at day 7 and 11.

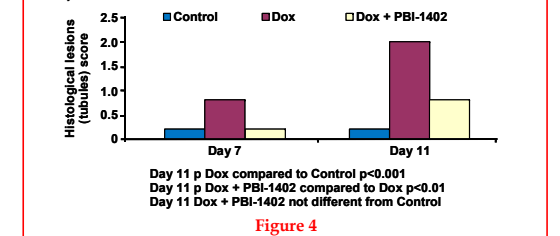


Figure 4

TGF- β is a well-known pro-fibrotic cytokine. CTGF is a down-stream mediator of TGF- β which modulates renal fibrosis and epithelial-mesenchymal transition in progressive kidney disease. **Figures 5 and 6** represent the effect of treatment of PBI-1402 on TGF- β and CTGF mRNA expression in the kidney. Dox induces TGF- β and CTGF mRNA expression in the kidney. Prophylactic treatment with PBI-1402 induces a significant ($p = 0.03$) reduction of the expression of TGF- β (29%) and CTGF (33%) in the kidney.

Effect of PBI-1402 on TGF- β mRNA expression in Dox-induced nephrotoxicity at day 11.

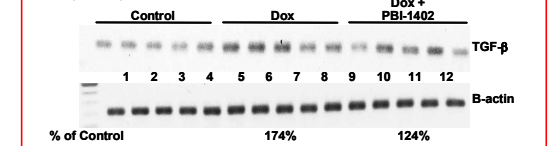


Figure 5

Effect of PBI-1402 on CTGF mRNA expression in Dox-induced nephrotoxicity at day 11.

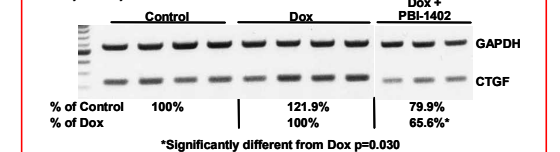


Figure 6

CONCLUSION

These results suggest that PBI-1402 has protective effects against Dox-induced nephrotoxicity in the acute phase of toxicity and encourage further studies regarding its use as a potential nephroprotective agent.

PBI-1402 preserves renal function by:

- ❖ \downarrow albuminuria
- ❖ \downarrow histological damage
- ❖ \downarrow TGF- β and CTGF expression: anti-fibrosis effect