# Bortezomib attenuates liver fibrosis and portal hypertension – underlying mechanisms

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## Introduction

Cirrhosis is prevalent world-widely and represents a high global burden of disease[1]. However, current treatment for cirrhosis is mainly focused on improving symptoms and preventing complications such as those due to portal hypertension, and there is currently no effective causal treatment for advanced fibrosis or cirrhosis. Bortezomib (B) is a reversible inhibitor of the chymotrypsin-like domain of the 20S proteolytic core within the 26S proteasome, and is currently used to treat malignancy, especially multiple myeloma[2].

## Aim

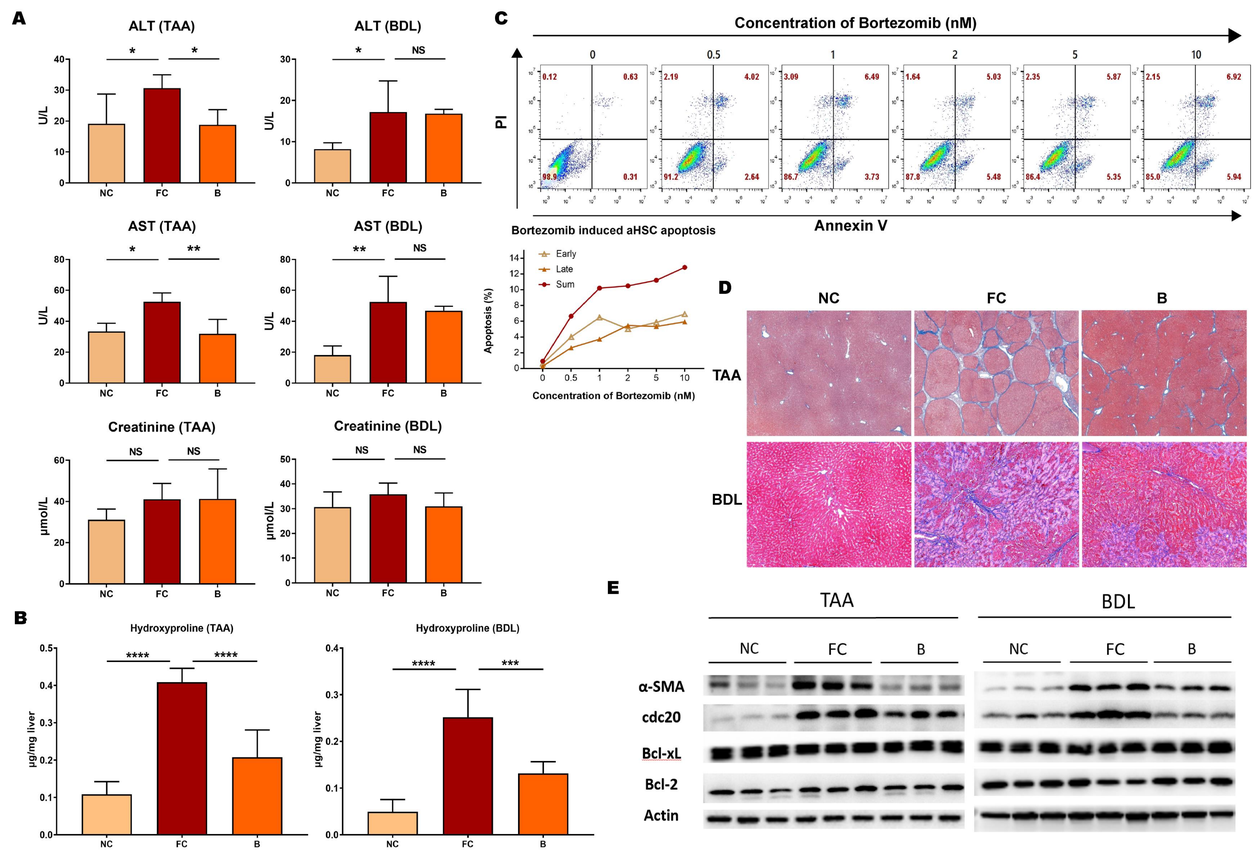
Bortezomib has been shown to attenuate fibrosis of skin, lung and kidney. We therefore aimed to evaluate the efficacy of B to improve advanced parenchymal and biliary liver fibrosis in rats and potential underlying mechanisms.

## Methods

Advanced liver fibrosis was induced in 8-week-old rats by intraperitoneal injection of thioacetamide (TAA) for 12 weeks or bile duct ligation (BDL) for 3 weeks. Rats with advanced fibrosis were divided into 2 groups: intravenous injection of B at 0.1 mg/kg in PBS (B) or injection of PBS alone three times a week for 3 weeks (fibrotic controls, FC). Age matched rats with no intervention served as normal controls (NC). Portal vein pressure was measured after anesthesia before sacrifice. Liver and renal function parameters were determined to evaluate liver inflammation and potential toxicity of B. HE and Masson staining were used to evaluate liver inflammation and fibrosis. Liver collagen was quantified via hydroxyproline content. Fibrosis-related gene and protein expression were determined by RT-qPCR and Western blot. Liver samples were subjected to NGS analysis. LX-2 human hepatic stellate cells and LO2 human hepatocytes were cultured with different concentrations of B for 24 h. Cell apoptosis was quantified via flow cytometry.

## Results

Portal vein pressure (mmHg) showed a decrease after B treatment, though the p-values did not yet reach statistical significance in the two fibrosis models (B vs FC: TAA, 6.86 ± 0.76 vs 11.42 ± 2.37; BDL, 9.49 ± 2.92 vs 18.57 ± 16.83). ALT and AST decreased with B treatment in both fibrosis models, and serum creatinine and bilirubin levels remained unchanged, indicating a beneficial effect of B on liver function with no apparent liver or renal toxicity **(A)**. Morphometry and biochemical quantification showed a significant amelioration of fibrosis, as evidenced by liver hydroxyproline content and Masson-positive area **(B)**. Gene set enrichment analysis indicated the upregulation of pro-inflammation-related ne *Ugt2b7* and anti-proliferation-related gene *Marvled1*, and downregulation of *Cdc20*, which is a key regulator in ubiquitin-mediated proteolysis and cell cycle pathways upon treatment with B. Cdc20 protein was down-regulated in B-treated rats **(C)**. B-treated hepatic stellate cells but not hepatocytes showed prominent apoptosis **(D)**.




***(A)** Liver and renal function parameters in rats. **(B)** Hydroxyproline determination. **(C)** Apoptosis of LX-2 human hepatic stellate cells treated by B in different concentrations. **(D)** Masson staining of rats' livers. **(E)** Western blot detection of CDC20, and fibrosis-related and apoptosis-related targets in rat liver samples.*

## Conclusion

Bortezomib has a prominent antifibrotic effect in parenchymal and biliary fibrosis by inducing activated hepatic stellate cell apoptosis

### References

1 **Gines P et al**. Liver cirrhosis. *Lancet 2021;398;:1359-1376.*
2 **Tan CRC et al**. Clinical Pharmacokinetics and Pharmacodynamics of Bortezomib. *Clin Pharmacokinet 2019;58;:157-168.*

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