Modulation of GPBAR1 attenuates liver fibrosis in a mouse model of autoimmune cholestatic liver disease

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Background

Primary biliary cholangitis (PBC) and primary sclerosing cholangitis (PSC) represent the main forms of autoimmune cholestatic liver characterized by chronic inflammation and progressive bile ducts loss. The etiology of PBC and PSC results from complex interactions between a genetic background, environmental trigger and immune dysregulation. Macrophages play a role in the pathogenesis of autoimmune cholestasis at the interface between the innate and adaptive immunity. The GPBAR1 (also known as TGR5) is a cell membrane receptor for secondary bile acids. GPBAR1 is highly expressed in Kupffer cells, liver endothelial cells and cholangiocytes and its expression is reduced PBC patients. Whether GPBAR1 plays a mechanistic role in PBC, however, remains poorly defined. BAR501 is a GPBAR1 agonist that exerts anti-inflammatory effects.

Materials and Methods

In vitro assays were performed on a normal human cholangiocytes (NHC) cell and U937, a monocyte line. In vivo, Abcb4 -/- male mice were administered with BAR501 (10 mg/Kg) for 16 weeks. ANIT model on Il10-/- mice adminestred with BAR501 (10 mg/Kg).

Results

GPBAR1 expression, mRNA and protein, was detected in NCH, and its activation with BAR501 (10 μM) reverted the pro-inflammatory phenotype promoted by LPS (100 ng/ml) as demonstrated by downregulation of pro-inflammatory markers (Figure 1). Administered in vivo to Abcb4$^{-/-}$, BAR501 attenuated hepatic damage as documented by reduced of AST, ALT and bilirubin plasma levels and the number of plasmatic WBC counts (Figure 2A, B). BAR501 reduced the severity of liver fibrosis and expression of the Col1a1 and αSMA mRNAs (Figure 2G). Moreover, BAR501 decreased the CDK19 positive area, as well the expression of Il-6 and Gpbar1 mRNAs (Figure 2F, H and I). RNAseq analysis of liver samples reveal major dissimilarities between Abcb4$^{-/-}$ left untreated or administered with BAR501, down-regulating the expression of many genes belonging to inflammatory and pro-fibrotic pathways (Figure 3). BAR501 reversed the developed of a pro-inflammatory phenotype induced by challenging U937 cells with NHC supernatants (LPS induction), reversing the M1 phenotype and IL-6 production (Figure 4).

Conclusions

GPBAR1 agonism, counter-regulates the development of a pro-inflammatory phenotype of liver macrophages, this reducing development of inflammation, fibrosis and bile duct damage in models of cholestasis.

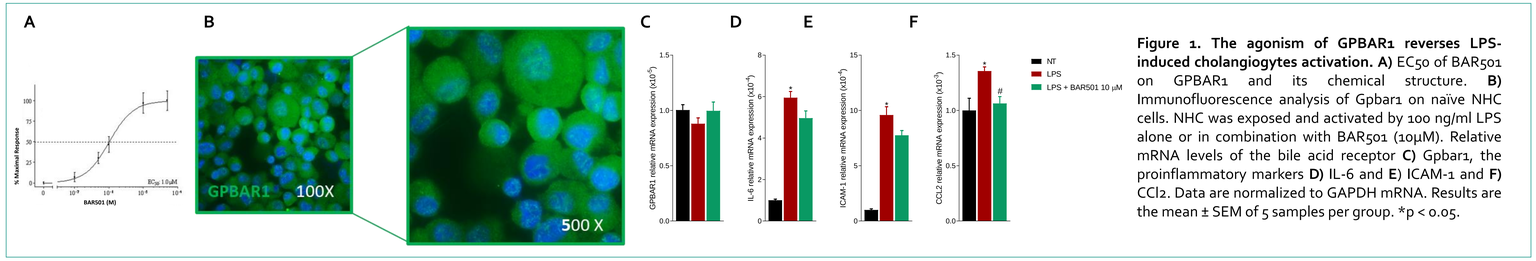

Figure 1. The agonism of GPBAR1 reverses LPS-induced cholangiogytes activation. A) EC50 of BAR501 on GPBAR1 and its chemical structure. **B)** Immunofluoroescence analysis of Gpbar1 on naïve NHC cells. NHC was exposed and activated by 100 ng/ml LPS alone or in combination with BAR501 (10μM). Relative mRNA levels of the bile acid receptor **C)** Gpbar1, the proinflammatory markers **D)** IL-6 and **E)** ICAM-1 and **F)** CCl2. Data are normalized to GAPDH mRNA. Results are the mean ± SEM of 5 samples per group. *p < 0.05.

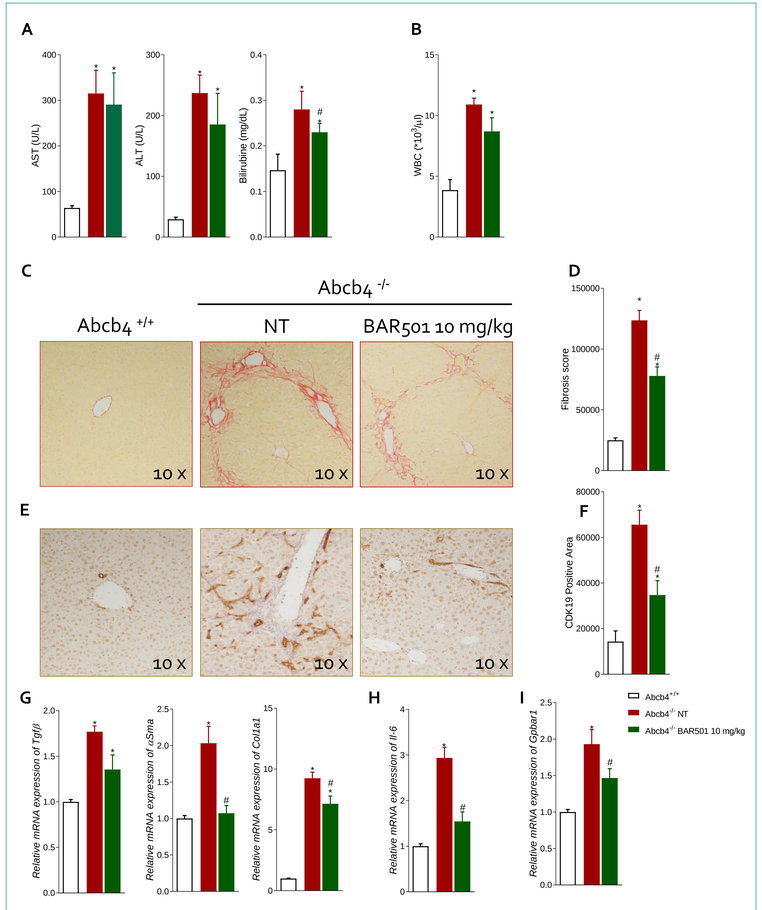

Figure 2. BAR501 protects against the development of liver damage and fibrosis in Abcb4-/- male mice Abcb4-/- male mice spontaneously develop biliary damage and liver fibrosis. They were administered with BAR501 at the dose 10 mg/Kg/daily for 16 weeks. Data shown are **A)** Plasma levels of AST (U/L), ALT (U/L)and Bilirubine (mg/dL). **B)** Number of plasmatic WBC counts. **C)** Syrius-Red staining of liver sections (10x Magnification) with **D)** fibrosis score in arbitrary units. **E)** CDK19 IHC staining on liver sections (10x Magnification) with **F)** CDK19 positive calculation in arbitrary units. Relative mRNA expression of **G)** fibrosis marker genes: Tgfβ, αSma and Col1a1; **H)** the proinflammatory cytokine Il-6; and BAR501 target **I)** Gpbar1. Data are normalized to Gapdh mRNA. Results are the mean ± SEM of 3 mice per group. (*p < 0.05).

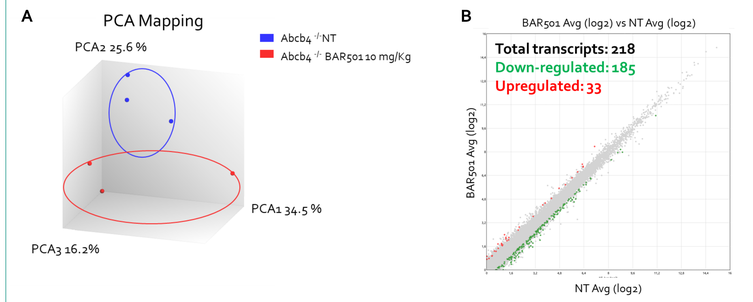

Figure 3. RNA-seq analysis of Abcb4 -/- liver left untreated or administrated with 10 μM of BAR501. A) Heterogeneity characterization of the two experimental groups as shown by principal component analysis (PCA) plot. **B)** Scatter plots of transcripts differentially expressed between different experimental groups (fold change <−2 or >+2, p value < 0.05). Red dots represent significantly upregulated genes, and green dots represent significantly downregulated genes.

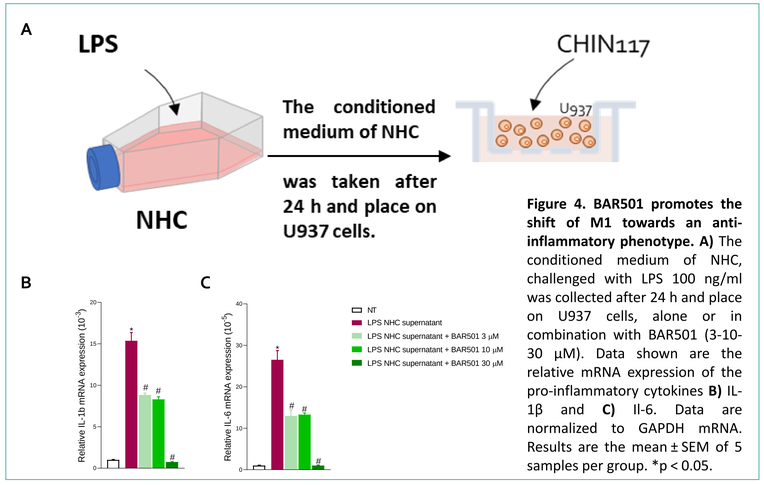

Figure 4. BAR501 promotes the shift of M1 towards an anti-inflammatory phenotype. A) The conditioned medium of NHC, challenged with LPS 100 ng/ml was collected after 24 h and place on U937 cells, alone or in combination with BAR501 (3-10-30 μM). Data shown are the relative mRNA expression of the pro-inflammatory cytokines **B)** IL-1β and **C)** Il-6. Data are normalized to GAPDH mRNA. Results are the mean ± SEM of 5 samples per group. *p < 0.05.

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