2143: GNS561, A NEW LYSOSOMOTROPIC SMALL MOLECULE, FOR THE TREATMENT OF LIVER FIBROSIS

C. Ansaldi[1], E. Bestion[1], J. Boursier[2], G. Lalmanach[3], M. Novello[1], Z. M. Jilkova[4,5,6], F. Bassissi[1], L. Vanderlynden[3], M. Rachid[1], L. Fizanne[2], J. Tracz[1], J. Courcambeck[1], C. Serdjebi[1], T. Decaens[4,5,6], P. Halfon[1] and S. Brun[1]

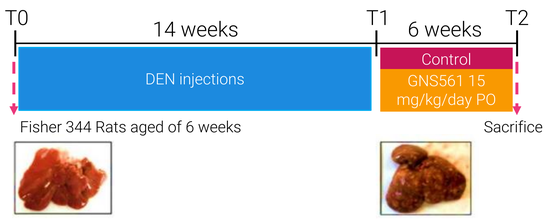
[1]Genoscience Pharma, Marseille, France, [2]Hepatology, Angers University Hospital, France, [3]Tours University, France, [4]Grenoble University, France, [5]Hepatology Department, Angers University Hospital, France, [6]Hepato-gastroenterology department, Grenoble Alpes University Hospital

Introduction

- Liver fibrosis is the final consequence of many chronic liver injuries. Hepatic fibrosis is a wound-healing response leading to massive accumulation of extracellular matrix (ECM) in liver. The non-functional ECM progressively replace the functional tissue and lead to a broad spectrum of complication such as liver failure, hepatocellular carcinoma and death.
- In liver injured tissue, activated hepatic stellate cells (HSC) are wildly recognized as the main cellular source of ECM compounds. They play a central key role in fibrogenesis and liver fibrosis development by regulating compounds such as TGF-β1 cytokine.
- To date, despite intensive research in this field, there are no anti-fibrotic drugs licensed for human use. In this context, we investigated the in vitro and in vivo anti-fibrotic activity of GNS561, a new small molecule orally available with high liver affinity tested in clinical trial (NCT03316222).

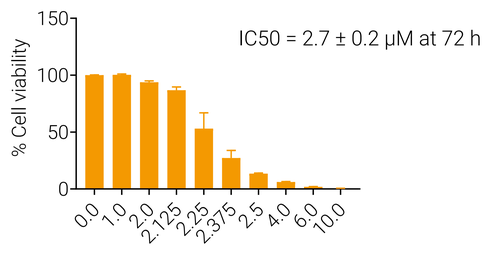
Methods

- Use of LX-2 cell line, which exhibit typical features of activated HSC in culture
- Measure of cell viability and caspase 3/7 activation using CellTiter Glo® and Caspases-Glo® 3/7 assays (Promega)
- Protein expression quantification and autophagic flux study by western-blotting and Image J analysis
- Determination of peptidase activity of cathepsin B (CTSB), both cathepsins B and L (CTSB/L), and cathepsin D (CTSD) by fluorometry using synthetic substrates
- Antifibrotic activity evaluation in a cirrhotic rat model of induced fibrosis obtained after 14 weekly intraperitoneal injections of diethylnitrosamine (DEN)

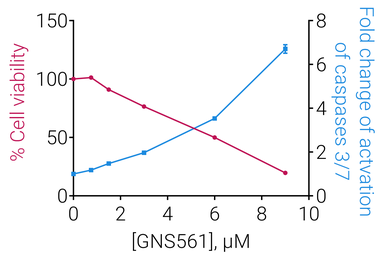


Results

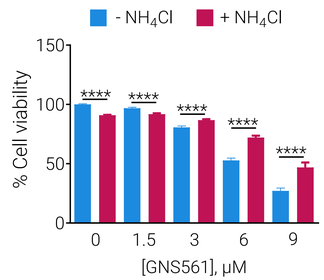
A **Reduction of HSC viability (LX-2, 24 h)**



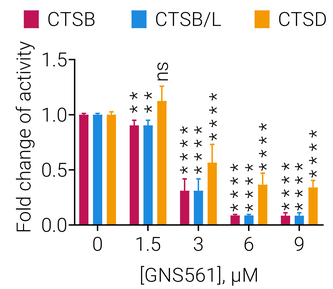
B **Induction of apoptosis via activation of caspases 3/7 (LX-2, 24 h)**

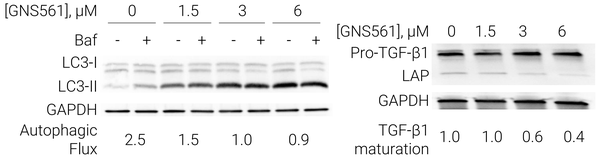


C **Essential lysosomotropic nature of GNS561 for its effect on fibrosis (LX-2, 24 h)**

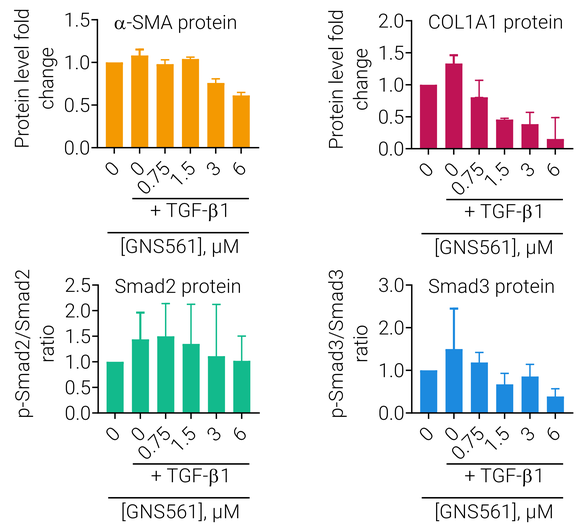


D **Decrease of lysosomal cathepsins (B, L and D) activity leading to an inhibition of autophagic flux and of TGF-β1 maturation (LX-2, 24 h)**

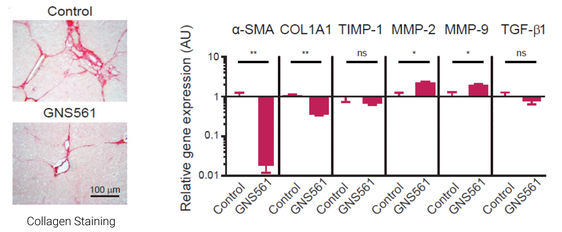




E **Decrease of HSC activation via inhibition of TGF-β1 / Smad signaling pathway (LX-2, 24 h, 2 h of stimulation with TGF-β1)**



F **Decrease of collagen deposition and pro-fibrotic markers and increase of fibrolysis in DEN-induced liver fibrosis rats model**



Conclusion

- GNS561 induces the apoptosis of HSC and also prevents HSC activation and decreases ECM deposition (reducing type I collagen synthesis and indirectly removing the scar tissue).
- GNS561 decreases cathepsin activity leading to: 1) weaker TGF-β1 maturation and the subsequent downregulation of the TGF-β1/Smad signaling pathway and 2) defective autophagy flux and a lack of energy.
- Abolition of GNS561-induced cell death by disruption of the lysosomal pH gradient confirmed that GNS561 lysosomotropism is responsible for its anti-fibrotic effect.
- Oral administration of GNS561 was well tolerated and attenuated DEN-induced liver fibrosis in this rat model.

GNS561 is a new compound that could represent a therapeutic option for hepatic fibrosis treatment through both its anti-fibrotic and pro-fibrolytic effects.

References & Contacts

EB, MN, FB, MR, JT, JC, CS, CA, PH and SB are employees of Genoscience Pharma. FB, CS, CA, PH and SB are shareholders of Genoscience Pharma. FB, MR, JC, PH and SB are co-authors of a pending patent of GNS561 in fibrosis

e.bestion@genosciencepharma.com