NITAZOXANIDE DIRECTLY PROTECTS FROM STRESS-INDUCED CELL DEATH TO ALLEVIATE LIVER DAMAGE IN PRECLINICAL MODELS OF ACUTE-ON-CHRONIC LIVER FAILURE

Marie Bobowski-Gerard[1], Simon Debaecker[1], Nicolas Stankovic Valentin[1], Alexia Tessier[2], Hana El Khatib[1], Philippe Delataille[1], Remy Hanf[3], Sakina Sayah Jeanne[1], Dean W. Hum[1], Vanessa Legry[1], Bart Staels[2]

[1]GENFIT SA, Loos, France; [2]Univ. Lille, Institut Pasteur de Lille, CHU Lille, Inserm U1011, Lille, France; [3]Pradial-consulting, Lille, France

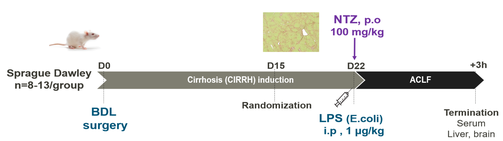
BACKGROUND & AIM

Acute-on-chronic liver failure (ACLF) is characterized by an acute decompensation of liver function in patients with cirrhosis; associated with multiple organ failures and high short-term mortality rates. We previously showed that the anti-parasitic drug nitazoxanide (NTZ) counteracts LPS-induced inflammatory responses and organ failures in disease models of ACLF[1,2].

- **To further assess the direct effect of NTZ on liver injury**
- **NTZ and its active circulating metabolite tizoxanide (TZ) were assessed on apoptosis and necroptosis, two modes of cell death reported to contribute to ACLF pathophysiology[3,4], using animal and cellular models of ACLF.**

MATERIAL & METHODS

ACLF induction in rat with cirrhosis



Transcriptomic analysis

- Liver mRNA were extracted for RT-qPCR analyses (CFX96 Touch™) or Illumina RNA-sequencing. Differentially expressed genes were retrieved as described in [2]. Enrichment analyses were performed with Gene Set Enrichment Analysis (GSEA) and cell death pathways as gene sets (merged from Reactome, IPA, KEGG and Wikipathway). The Normalized Enrichment Score (NES) and adjusted p-value (FDR) were retrieved for each pathway.

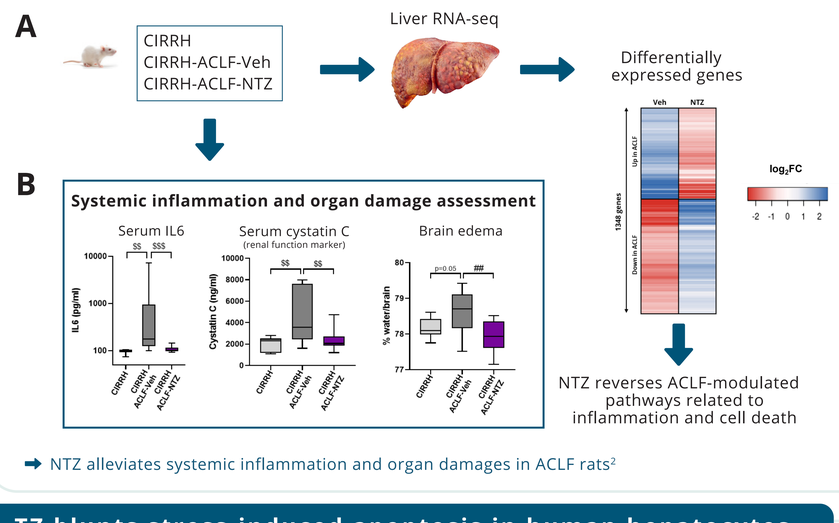
Evaluation of TZ in cellular models of cell death

- For apoptosis induction, human immortalized hepatocytes (IHH)[5] were stimulated with H_2O_2 or ethanol. TZ or vehicle was added concomitantly (co-treatment) or one hour after stimulation (post-treatment).
  Caspase 3/7 activity in cell lysates was measured through the Caspase-Glo® 3/7 luminescent assay (Promega). For Western blot analysis, protein extracts were analyzed through a Jess system (Protein simple™), using cleaved caspase 3 (c-casp3) antibody (#9664, Cell Signaling) and β-actin antibody (#MAB8929, R&D system).
- Necroptotic cell death was induced in human colon cancer cells HT-29 using a combination of the pan-caspase inhibitor zVAD (10 µM), the Smac mimetic BV6 (2 µM) and TNF-alpha (20 ng/mL). LDH release was measured in the supernatant through the LDH-glo™ cytotoxicity assay (Promega).

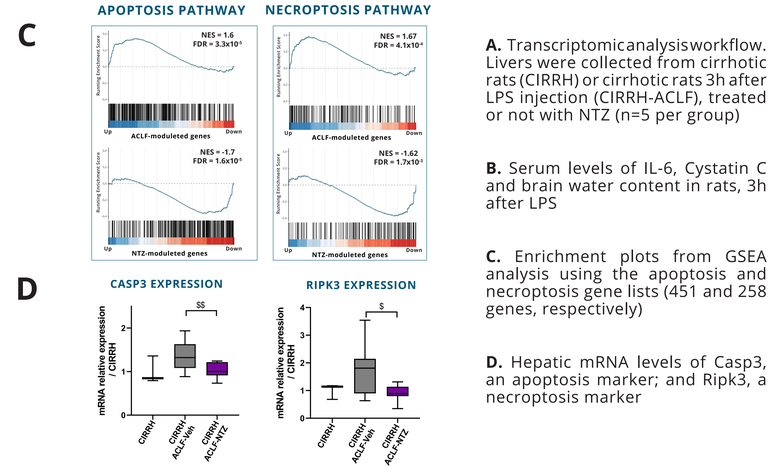
STATISTICAL ANALYSES

Box plots show the median as a line, 25th to the 75th percentile as a box and min and max as whiskers
For data following a normal distribution: #: p<0.05; ##: p<0.01; ###: p<0.001 (two-tailed Student T test), *: p<0.05; **: p<0.01; ***: p<0.001 (One-way Anova)
For non-normally distributed variables: $: p<0.05; $$: p<0.01; $$$: p<0.001 (two-tailed Mann-Whitney), §: p<0.05; §§: p<0.01; §§§: p<0.001 (Kruskall-Wallis)
For RNA-seq analysis, Anova and Kruskal-Wallis tests, the p-values were adjusted using Benjamini-Hochberg correction.

NTZ modulates cell death-associated gene signature in an ACLF rat model



➡ NTZ alleviates systemic inflammation and organ damages in ACLF rats[2]



➡ NTZ reverses apoptosis and necroptosis pathways in ACLF rat livers

A. Transcriptomic analysis workflow. Livers were collected from cirrhotic rats (CIRRH) or cirrhotic rats 3h after LPS injection (CIRRH-ACLF), treated or not with NTZ (n=5 per group)

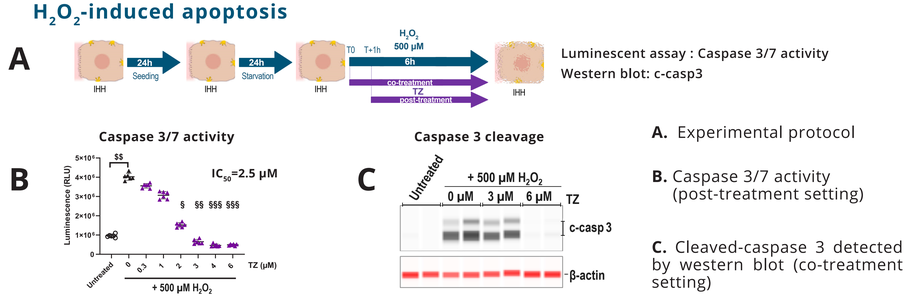
B. Serum levels of IL-6, Cystatin C and brain water content in rats, 3h after LPS

C. Enrichment plots from GSEA analysis using the apoptosis and necroptosis gene lists (451 and 258 genes, respectively)

D. Hepatic mRNA levels of Casp3, an apoptosis marker; and Ripk3, a necroptosis marker

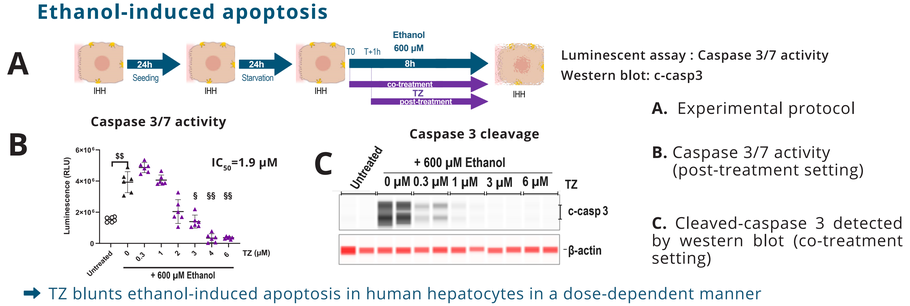
TZ blunts stress-induced apoptosis in human hepatocytes

H_2O_2-induced apoptosis



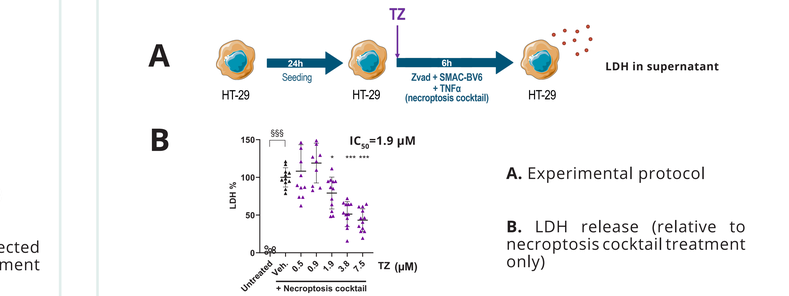
➡ TZ blunts H_2O_2-induced apoptosis in human hepatocytes in a dose-dependent manner

Ethanol-induced apoptosis



➡ TZ blunts ethanol-induced apoptosis in human hepatocytes in a dose-dependent manner

TZ reduces toxicity in a model of necroptosis



➡ TZ inhibits LDH release induced by a necroptotic stimuli in a dose-dependent manner

CONCLUSION

Direct protective effects of TZ on stress-induced apoptosis and necroptosis identifies NTZ treatment as a promising approach to protect the liver of patients with ACLF from cell death.

REFERENCES & ACKNOWLEDGMENT

1. Legry et al. Journal of Hepatology, 2022 vol. 77, THU528; **2.** Legry et al., Journal of Hepatology, 2023 vol. 78, THU-342, **3.** Adebayo et al., Liver Int., 2015, 35(12):2564-25744; **4.** Kondo et al., Cell Death Dis., 2021, 13(1):5; **5.** Schippers et al., Cell Biol Toxicol. 1997, 13(4-5):375-386; **6.** Abd-Elsalam et al. J Clin Gastroenterol 2019 Vol.53: 226-230; **7.** Glal et al. J Hepatobiliary Pancreat Sci. 2021 Vol.28:812-824, **8.** Dubreuil et al. Antimicrob Agents Chemother 1996 Vol.40:2266–2270, **9.** Musher et al. Clinical Infectious Diseases 2006 Vol.43:421-427, **10.** Pankuch et al. Antimicrob Agents Chemother. 2006 Vol.50(3):1112-7

We thank Nina T'Serstevens and Constance Bonhomme for their contribution to this work.