NITAZOXANIDE COUNTERACTS LIPOPOLYSACCHARIDE-INDUCED HEPATIC AND RENAL TRANSCRIPTOMIC PROFILE CHANGES TO IMPROVE SYSTEMIC INFLAMMATION AND ORGAN DAMAGE IN A DISEASE MODEL OF ACUTE-ON-CHRONIC LIVER FAILURE

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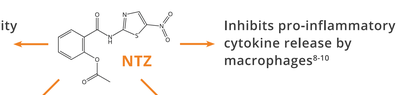
BACKGROUND

Acute-on-chronic liver failure (ACLF) is characterized by systemic inflammation and multiple organ failures in patients with cirrhosis and is associated with high short-term mortality. Among the precipitating factors, bacterial infection or translocation from the gut is the most important trigger in ACLF. The ensuing systemic inflammation in patients with cirrhosis results in an uncontrolled cytokine storm which induces cell death and jeopardizes the functioning of vital organs such as the liver and the kidney. The FDA-approved anti-parasitic drug nitazoxanide (NTZ) has shown promising effects in disease models of systemic inflammation and ACLF.

Rationale for repurposing nitazoxanide (NTZ) in ACLF



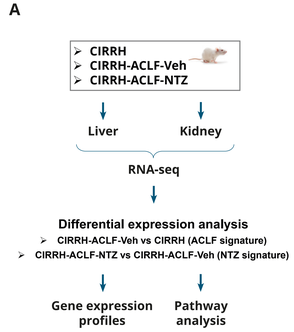
- Broad antimicrobial activity against Gram+ and Gram- intestinal bacteria[1-7]
- Inhibits pro-inflammatory cytokine release by macrophages[8-10]
- Improves hepatic and renal function markers in ACLF rats[8]
- Protects from encephalopathy in patients with cirrhosis[11,12]

AIM

To characterize the mechanism of action of NTZ, transcriptomic analyses were performed on liver and kidney from ACLF rats. The effect of NTZ on systemic inflammation and organ damages was also assessed.

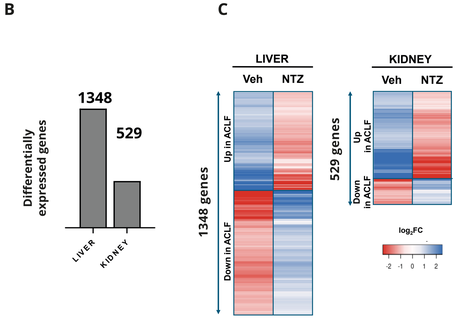
NTZ RAPIDLY REVERSES HEPATIC, RENAL AND SYSTEMIC INFLAMMATORY RESPONSE IN ACLF

Transcriptomic analysis workflow



A - Organs were collected in cirrhotic rats (CIRRH) or in cirrhotic rats 3h after LPS injection (CIRRH-ACLF), treated or not with NTZ (n=5 per group).

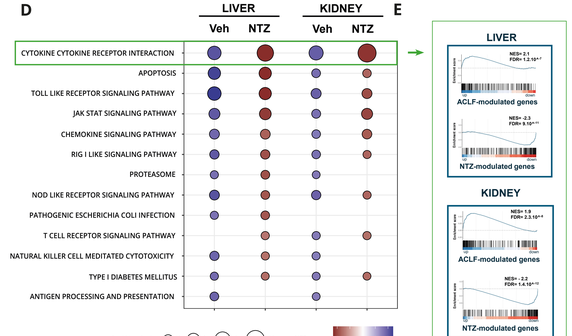
NTZ reverses the ACLF-induced gene signature



B - Number of differentially expressed genes between CIRRH-ACLF-Veh and CIRRH rats (Fold-changes >2, FDR<0.01)

C - Heatmap displaying (log2) fold-changes of ACLF-modulated genes (Veh) and their modulation by NTZ treatment

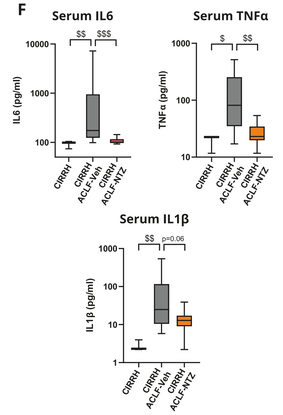
NTZ reverses the ACLF-modulated pathways



D - Bubble map displaying GSEA analysis using KEGG pathways as gene set. Colors indicate the Normalized Enrichment Score (NES) while dot areas are inversely proportional to the false discovery rate (FDR).

E - Enrichment plots from GSEA analysis using the cytokine-cytokine receptor interaction pathway as example (265 genes)

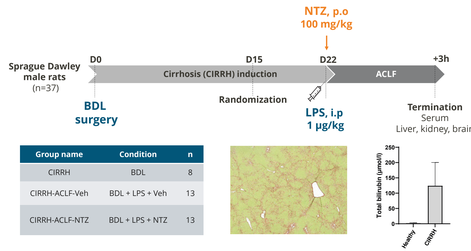
NTZ inhibits ACLF-induced circulating cytokines



F - Cytokines measured in serum 3h after ACLF induction

MATERIAL & METHODS

ACLF induction in rats with cirrhosis: Male Sprague Dawley rats underwent **bile duct ligation (BDL)** to induce cirrhosis. Twenty-two days after BDL surgery, ACLF was induced by intraperitoneal (ip) injection of 1 µg/kg LPS (*Escherichia coli* O111:B4). NTZ (100 mg/kg) or vehicle were orally administered concomitant to LPS injection. Serum and liver tissue samples were collected 3 hours after LPS injection. Cirrhosis induction was validated through Sirius red staining of liver section and elevated serum total bilirubin.



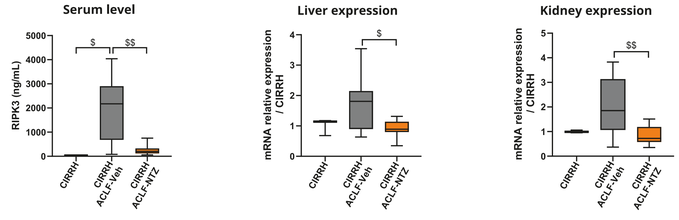
Group name	Condition	n
CIRRH	BDL	8
CIRRH-ACLF-Veh	BDL + LPS + Veh	13
CIRRH-ACLF-NTZ	BDL + LPS + NTZ	13

Serum and tissue analyses: Serum levels of cytokines were measured by Luminex. Cystatin C and RIPK3 serum levels were measured by ELISA. To evaluate brain edema, fresh brains were weighed, dehydrated and weighed again to calculate the percentage of water. High-quality mRNA was extracted from liver and kidney tissues for RT-qPCR analyses (CFX96 Touch™) or Illumina RNA-sequencing.

Transcriptomic analyses: Reads quality was analyzed using FastQC and trimmed when necessary. After mapping to the rat genome (BN7.2), DEseq2 was used to retrieve differentially expressed genes. Enrichment analyses were performed with Gene Set Enrichment Analysis (GSEA) and 181 KEGG pathways as gene sets. The Normalized Enrichment Score (NES) and adjusted p-value (FDR) were retrieved for each pathway.

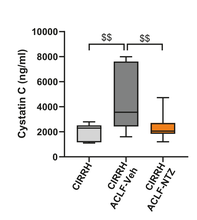
NTZ ALLEVIATES HEPATIC, RENAL AND CEREBRAL DAMAGE IN ACLF

Ripk3 necroptosis marker



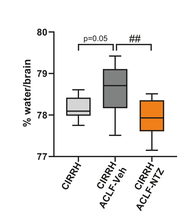
- NTZ lowers ACLF-induced serum, hepatic and renal levels of Ripk3, suggesting a protective effect of NTZ on necroptosis, a pro-inflammatory type of cell death important in ACLF[13]

Serum cystatin C



- NTZ improves ACLF-induced cystatin C, a renal function marker

Cerebral edema



- NTZ prevents cerebral edema of ACLF rats

STATISTICAL ANALYSIS

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

REFERENCES

1. Dubreuil *et al.* Antimicrob Agents Chemother 1996 Vol.40:2266–2270; **2.** Hecht *et al.* Antimicrob Agents Chemother 2007 Vol.51:2716–2719; **3.** Hoffman *et al.* Antimicrob Agents Chemother 2007 Vol.51:868–876; **4.** MacVay *et al.* Antimicrob Agents Chemother 2000 Vol.44: 2254-2258; **5.** Megraud *et al.* Antimicrob. Agents Chemother. 1998 Vol. 42:2836-2840; **6.** Musher *et al.* Clinical Infectious Diseases 2006 Vol.43:421-427; **7.** Pankuch *et al.* Antimicrob Agents Chemother. 2006 Vol.50(3):1112-7. **8.** Legry *et al.* Journal of Hepatology 2022 vol. 77, THU528 **9.** Hong *et al.* Int Immunopharm 2012 Vol.13:23-27; **10.** Shou *et al.* Inflammation 2019 Vol. 42:1336–1349; **11.** Abd-Elsalam *et al.* J Clin Gastroenterol 2019 Vol.53: 226-230; **12.** Glal *et al.* J Hepatobiliary Pancreat Sci. 2021 Vol.28:812-824 **13.** Kondo *et al.*, Cell Death Dis 2021 13(4):416; **14.** Harisseh *et al.* Digestive Disease Week 2023 (Chicago, USA) Abstract Tu1503

CONCLUSION

- **A single dose of NTZ rapidly counteracts the ACLF-associated gene signature in liver and kidney**
- **The anti-inflammatory activity of NTZ is associated with improved organ function in ACLF**
- **These data are in line with the results of the clinical Phase 1 study (NCT05116826): 7-day treatment with NTZ 500 mg BID showed a trend in IL-6 and total bilirubin reduction in patients with severe hepatic impairment[14]**

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