# NTZ ALLEVIATES STRESS-INDUCED HEPATOCYTE CELL DEATH THROUGH MODULATION OF OXIDATIVE STRESS AND DNA DAMAGE SIGNALING PATHWAYS IN ACLF MODELS

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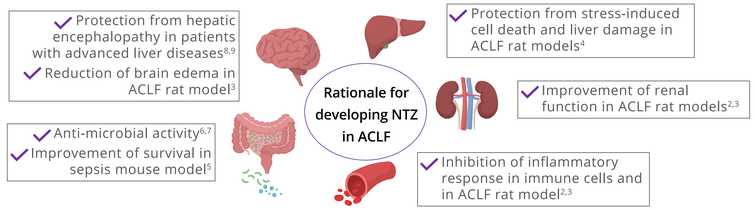
## BACKGROUND & AIM

- Nitazoxanide (NTZ) is an FDA approved anti-parasitic drug that is currently being investigated for the treatment of Acute-on-Chronic Liver Failure (ACLF), a severe syndrome affecting patients with acutely decompensated cirrhosis[1]
- We previously demonstrated that NTZ alleviates systemic inflammation and organ damage in disease models of ACLF[2-5]




- In human hepatocytes, tizoxanide (TZ, the active metabolite of NTZ), blunted stress-induced apoptosis in a dose-dependent manner. Moreover, NTZ modulated cell-death associated gene signature in the liver of a BDL+LPS rat model[4]

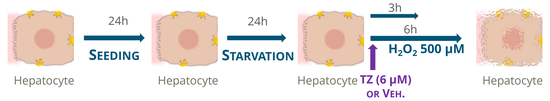
**To decipher the molecular mechanisms by which NTZ and TZ protect hepatocytes from cell death and liver damage, we performed transcriptomics analyses in two preclinical models:**

- ***In vitro*: human hepatocytes stressed with $H_2O_2$**
- ***In vivo*: liver from fibrotic rats who underwent LPS challenge (ACLF model)**

## METHODS & STATISTICS

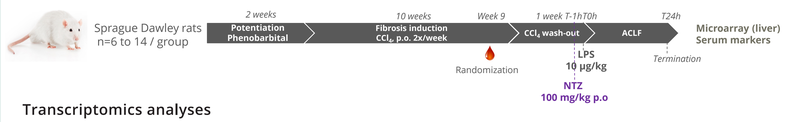
### Evaluation of TZ in human hepatocytes stressed with $H_2O_2$

Immortalized human hepatocytes were stressed with 0.5 mM $H_2O_2$, a method commonly used to trigger cellular oxidative damage and cell death. Tizoxanide (TZ ,6 µM) was added concomitantly with $H_2O_2$ for 3 or 6h. Caspase 3 cleavage was assessed by Western blot analysis




### Evaluation of NTZ in ACLF rats

Male Sprague Dawley rats received phenobarbital for 2 weeks then $CCl_4$ p.o. (uptitration to 0.85 mL/kg) twice a week for 10 weeks. LPS (10 µg/kg, i.p.) was injected one week after the last $CCl_4$ dose. NTZ (100 mg/kg) or vehicle was administered by oral gavage 1h before LPS injection. Surviving rats (17/27 in vehicle and 10/21 in NTZ group, respectively) were euthanized 24h after LPS injection. Serum markers of liver injury and function were measured using the Daytona plus automate




### Transcriptomics analyses

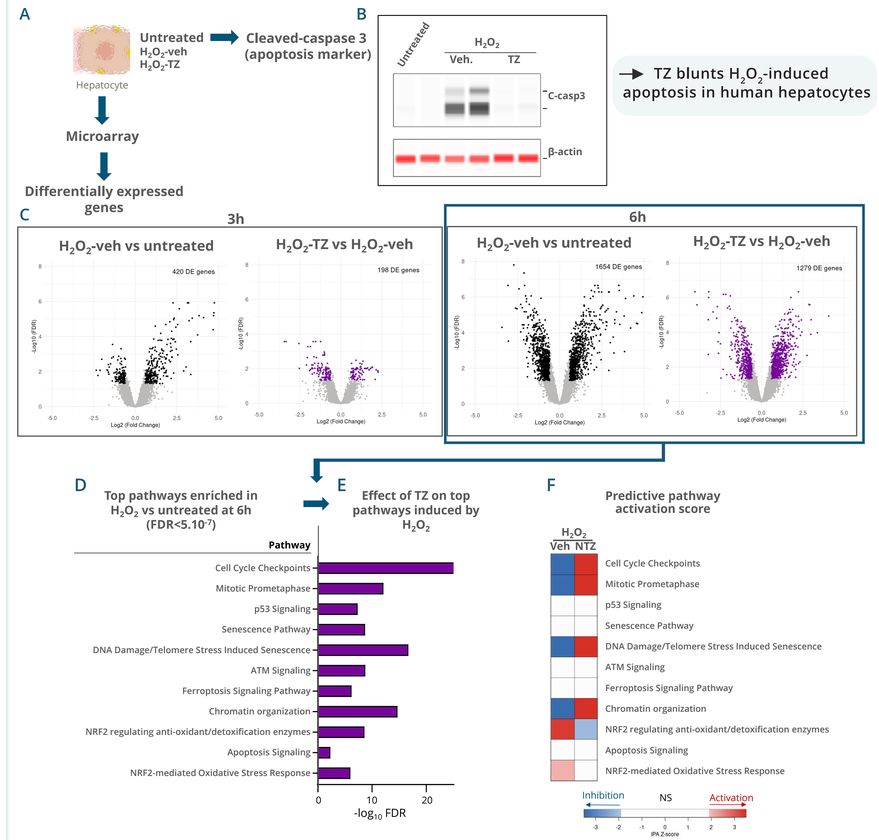
Hepatocytes and whole liver mRNA were extracted for RT-qPCR analyses (CFX96 Touch™) or for oligonucleotide microarray (GeneChipTM System 3000). Differentially expressed (DE) genes were identified using TAC software (Qiagen) followed by Limma analysis. Pathway analysis was performed with IPA (Qiagen)

### Statistical analysis

For microarray, 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 for non-normally distributed variables

## RESULTS

### TZ ALLEVIATES $H_2O_2$-INDUCED APOPTOSIS IN HUMAN HEPATOCYTES THROUGH MODULATION OF OXIDATIVE STRESS, DNA DAMAGE AND CELL CYCLE PATHWAYS



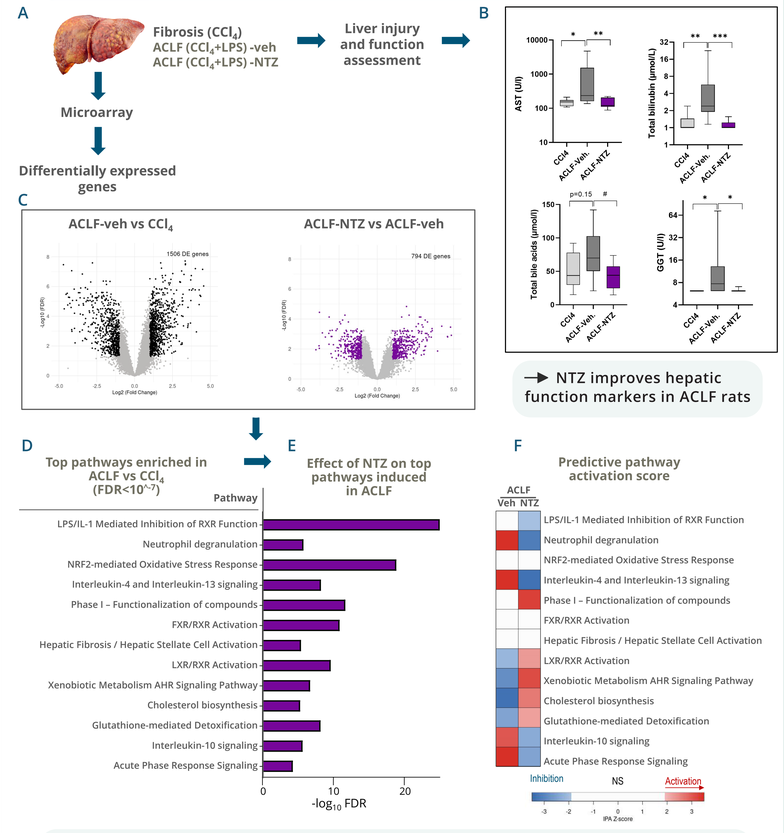

→ TZ blunts $H_2O_2$-induced apoptosis in human hepatocytes

→ $H_2O_2$-induces a transcriptomic remodeling, which is more pronounced after 6h $H_2O_2$ stimulation. $H_2O_2$–modulated genes are mostly related to cell cycle, DNA damage, cell death and oxidative stress

→ TZ strongly modulates the $H_2O_2$-induced gene signature, by reversing the effect of $H_2O_2$

*A. Experimental workflow (n=3 / group)*
*B. Cleaved caspase 3 expression in hepatocytes stressed with $H_2O_2$ for 6h*
*C. Volcano plots displaying significant DE genes (FC>1.5 and FDR<0.05) between $H_2O_2$ vs untreated groups (black dots) and between $H_2O_2$ -TZ vs $H_2O_2$ -veh groups (purple dots) after 3h or 6h $H_2O_2$.*
*D. Top enriched pathways using the list of DE genes between $H_2O_2$ -veh and untreated group at 6h (1654 genes)*
*E. Enrichment analysis using the list of DE genes obtained between $H_2O_2$ –NTZ and $H_2O_2$ -veh groups at 6h (1279 genes)*
*F. Activation score predicted by IPA using pathways analysis described in D. and E. Non significant (NS) Z-score (-2 to 2) are displayed in white*

### NTZ MODULATES LPS-INDUCED GENE SIGNATURE IN ACLF RAT LIVER, THEREBY PROTECTING FROM HEPATIC DAMAGE




→ NTZ improves hepatic function markers in ACLF rats

→ LPS induces a strong remodeling of fibrotic rat liver transcriptome, ACLF-modulated genes being mostly related to immune response, oxidative stress and metabolism

→ NTZ further modulates the ACLF gene signature, by reversing the effect of LPS

*A. Experimental workflow (n=5 to 6/group)*
*B. Serum levels of hepatic injury / function markers 24h after LPS in fibrotic rats*
*C. Volcano plots displaying DE genes (FC>2 and FDR<0.05) between ACLF-veh vs $CCl_4$-veh groups (black dots) and between ACLF-NTZ vs ACLF-veh groups (purple dots)*
*D. Top enriched pathways using the list of DE genes between ACLF-veh and $CCl_4$ groups (1506 genes)*
*E. Enrichment analysis using the list of DE genes obtained between ACLF –NTZ and ACLF-veh groups (794 genes)*
*F. Activation score predicted by IPA using pathways analyses described in D. and E. Non significant (NS) Z-score (-2 to 2) are displayed in white*

## CONCLUSION

- **In two ACLF-related preclinical models, transcriptomic analyses show that NTZ counteracts the deregulation of pathways involved in immune response, metabolism and oxidative stress response, which could explain the protective role of NTZ against hepatocyte cell death and liver damage**
- **Combined with its anti-bacterial and anti-inflammatory activities, these findings further support the development of NTZ as a new therapeutic approach for ACLF**

## REFERENCES

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