EFFICACY OF NITAZOXANIDE (NTZ) ON SYSTEMIC INFLAMMATION AND ORGAN FUNCTION IN DISEASE MODELS OF ACUTE-ON-CHRONIC LIVER FAILURE (ACLF) WHEN ADMINISTERED POST-ACLF TRIGGER

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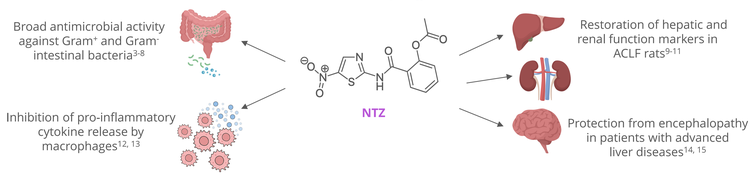
4165

BACKGROUND & AIM

- Acute-on-chronic liver failure (ACLF) is a life-threatening complication of cirrhosis, characterized by systemic inflammation and multi-organ dysfunction. Bacterial infections and translocation of pathogen-associated molecular patterns (PAMPs), such as lipopolysaccharide (LPS), are key drivers of disease onset and progression[1, 2]
- Nitazoxanide (NTZ) has demonstrated antimicrobial[3-8], anti-inflammatory[12, 13], and multi-organ protective[9-11, 14, 15] properties, and is currently under development for the treatment of ACLF (G1090N)
- In rodent disease models, we previously demonstrated the beneficial effect of NTZ on systemic inflammation and organ damage when added concomitantly or before the precipitating trigger of ACLF[9-11]
- The aim of this study was to better assess the activity of NTZ on preexisting inflammation, by investigating NTZ efficacy when administered after the pathological stimulus in preclinical models of inflammation and ACLF

Rationale for developing NTZ in ACLF



METHODS & STATISTICS

Evaluation of TZ on LPS-stimulated human PBMCs

Human Peripheral Blood Mononuclear Cells (PBMCs) were seeded for 1 h in culture medium with Fetal Bovine Serum (FBS), then cells were centrifuged and medium was changed to fresh serum-free medium. Inflammation was triggered by LPS 1 ng/mL (Escherichia coli O111:B4). Tizoxanide (TZ, the active metabolite of NTZ) was added to the PBMCs concomitantly or 30 min after LPS. The secretion of inflammatory cytokines was assessed in the extracellular medium through HTRF, 6 h post-trigger

Evaluation of NTZ in a rat model of LPS-induced endotoxemia

Endotoxemia was induced in healthy Sprague Dawley male rats by intraperitoneal (i.p.) injection of 1 mg/kg LPS (Escherichia coli O111:B4). A single oral dose of NTZ (pro-drug of TZ, 300 mg/kg) was administered concomitantly or 15 min after LPS. Serum was collected 3 and 5 h after LPS

Evaluation of NTZ in a rat model of ACLF (BDL + LPS)

Sprague Dawley male rats underwent bile duct ligation (BDL) to induce cirrhosis. Rats were stratified into treatment groups based on markers of hepatic fibrosis and functions measured 15 days after BDL surgery. ACLF was induced 22 days post-surgery by injection of LPS (Escherichia coli O111:B4, 1 µg/kg, i.p.). NTZ (300 mg/kg) or vehicle were orally administered 15 min after LPS injection. Serum was collected 3 h post LPS injection

Analysis of biochemical markers

Serum levels of cytokines were measured by Luminex. Serum hepatic and renal function markers were measured by Daytona plus automate. Cystatin C and RIPK3 serum levels were measured by ELISA

Statistical analysis

Experimental results are expressed as mean ± standard deviation and plotted as box plots or scatter plots. Box plots show the median as a line, 25th to the 75th percentile as a box and min and max as whiskers. Scatter plots show the mean as a line, and min and max as whiskers

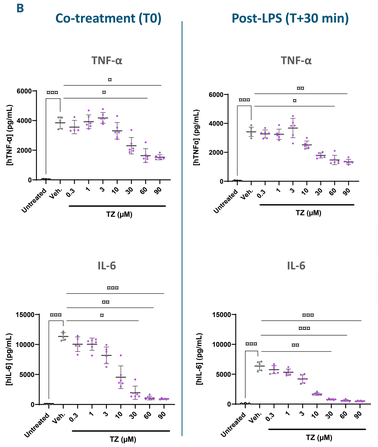
Normality for each parameter was assessed using the Shapiro-Wilk test or Kolmogorov-Smirnov test. For variables presenting a normal distribution, comparison between groups was tested using unpaired Student's t test (*: p<0.05; **: p<0.01; ***: p<0.001). A Welch's t test was applied for inequal variances (#: p<0.05; ##: p<0.01; ###: p<0.001). A one-way ANOVA test (§: p<0.05; §§: p<0.01; §§§: p<0.001) was used to compare LPS-TZ/NTZ-treated versus LPS-vehicle-treated groups. For other non-normally distributed variables, a non-parametric Mann-Whitney test was applied ($: p<0.05; $$: p<0.01; $$$: p<0.001). For the in vitro study, Kruskal-Wallis test with Dunn's multiple comparison was used (¤: p<0.05; ¤¤: p<0.01; ¤¤¤: p<0.001), and each concentration of compound was plotted using a non-linear regression to determine IC50. For some data, a log-scale was applied for better visualization

RESULTS

POST-CHALLENGE TZ ADMINISTRATION EFFECTIVELY BLUNTS CYTOKINE RELEASE BY HUMAN PBMCS

A. Experimental protocol
B. Secreted cytokines IL-6 and TNF-α in the extracellular medium

Untreated, n=3-7
LPS + Vehicle, n=5-6
LPS + TZ (0.3 to 90 µM), n=4-6



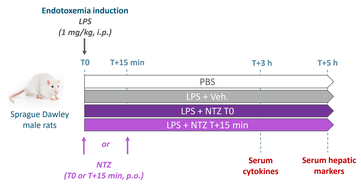
Comparison of efficacy in two TZ treatment settings:

		T0 Co-treatment	T+30 min Post-treatment
IL-6	IC_{50} (µM)	7.6	4.9
	Emax (%)	-92	-93
TNFα	IC_{50} (µM)	21	11
	Emax (%)	-56	-61

Legend:
- Untreated
- LPS + Vehicle (Veh.)
- LPS + TZ T0' or T+30 min

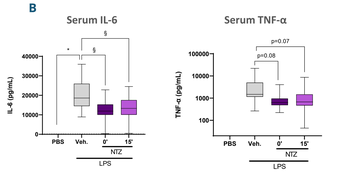
→ In human PBMCs, TZ reduced LPS-induced secretion of pro-inflammatory cytokines IL-6 and TNF-α with comparable efficacy when administered concomitantly or post-challenge

NTZ ADMINISTRATION POST-LPS MITIGATES SYSTEMIC INFLAMMATION AND IMPROVES LIVER FUNCTION

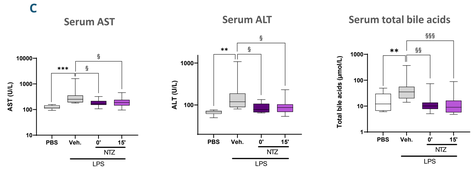


A. Experimental protocol
B. Circulating cytokines measured 3h post-LPS trigger
C. Serum levels of hepatic function markers measured 5h post-LPS trigger

Healthy (PBS-treated), n=8
Control (LPS + Vehicle), n=14
NTZ co-treatment (LPS + NTZ 0'), n=12
NTZ post-treatment T+15min (LPS + NTZ 15'), n=13

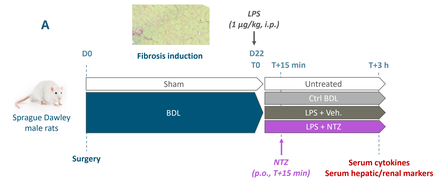


→ A single administration of NTZ, either simultaneously or shortly after endotoxemia induction, lowered systemic inflammation in rats



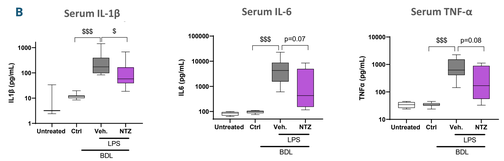
→ NTZ administration reduced hepatic injury markers in both treatment conditions in LPS-induced endotoxemia in rats

BENEFICIAL EFFECTS OF NTZ ON SYSTEMIC INFLAMMATION AND ORGAN FUNCTION IN A RAT MODEL OF ACLF

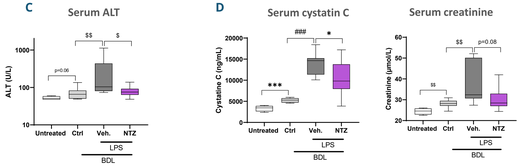


A. Experimental protocol
B. Circulating cytokines
C. Serum levels of hepatic function marker
D. Serum levels of renal function markers

Healthy (untreated), n=4
Cirrhotic (control BDL), n=8
ACLF (BDL + LPS + Vehicle), n=9
ACLF treated with NTZ (BDL + LPS + NTZ), n=11



→ Administration of NTZ after LPS administration reduced LPS-induced systemic inflammation, as shown by the reduction in circulating pro-inflammatory cytokines IL-1β, IL-6 and TNF-α



→ NTZ resolved liver injury, as shown by a decrease in hepatic function marker ALT

→ In addition, NTZ treatment reduced renal injury markers following LPS-induced ACLF in BDL rats

CONCLUSION

- **Tizoxanide, the active of metabolite of NTZ, lowered pro-inflammatory cytokine secretion post-LPS challenge *in vitro***
- **This rapid anti-inflammatory effect was also observed *in vivo* in a rat model of endotoxemia, as NTZ mitigated inflammation and lowered hepatic injury markers when administered post-challenge**
- **In a rat model of ACLF, a single dose of NTZ administered after LPS administration reduced systemic inflammation and restored hepatic and renal functions**
- **These findings further support the development of NTZ (G1090N) as a new therapeutic approach for ACLF**

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DISCLOSURE | MB, PD, SD, CV, DH, VL and SSJ are employees and stock shareholder of GENFIT S.A.; JT is consultant for GENFIT S.A.; BS is scientific adviser of GENFIT S.A.