DIFFERENTIAL ACTIVITY OF NITAZOXANIDE VS RIFAXIMIN IN LIPOPOLYSACCHARIDE-INDUCED DISEASE MODELS

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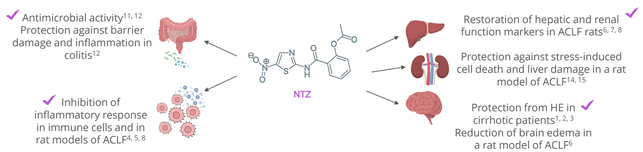
FRI-384

BACKGROUND & AIM

- Investigational drug G1090N, a new formulation of nitazoxanide (NTZ) is currently being developed for the treatment of acute-on-chronic liver failure (ACLF), a severe complication of acutely decompensated cirrhosis characterized by systemic inflammation and one or multiple organ failures. A Phase 1 clinical trial with G1090N (NCT07110441) confirmed favorable safety and tolerability profile
- NTZ is an antimicrobial compound with broad-spectrum activity that has shown promising effects on hepatic encephalopathy (HE) in three clinical studies[1, 2, 3]. Moreover, we demonstrated anti-inflammatory [4, 5] and organ protective activities of NTZ in disease models of ACLF [6, 7, 8]
- Rifaximin (RFX) is a poorly absorbed antibiotic, indicated for the prevention of hepatic encephalopathy (HE) recurrence in cirrhotic patients. The primary mechanism of RFX is thought to occur through its effects on the gut microbiome, although other potential beneficial activities, such as anti-inflammatory effects, have been reported[9, 10]

NTZ activities that could benefit ACLF patients:



To better understand the mechanisms of action of NTZ - and its main circulating active metabolite tizoxanide (TZ) - vs RFX, we compared their activities in acute models of inflammation induced by lipopolysaccharide (LPS), a key pathogen-associated molecular pattern (PAMP) in ACLF

- In vivo **study: evaluation of the effect of a single dose of NTZ or rifaximin in an LPS-induced endotoxemia rat model,**
- In vitro **study: assessment of the effect of TZ and RFX on LPS-induced cytokine release in human peripheral blood mononuclear cells (PBMCs)**

METHODS & STATISTICS

Evaluation of the efficacy of NTZ and RFX administered in a curative setting in a rat model of LPS-induced endotoxemia

- Sterile endotoxemia was induced in healthy rats by intraperitoneal (i.p.) injection of LPS (1 µg/kg). A single oral dose of NTZ (300 mg/kg) or rifaximin (50 mg/kg) was administered in a curative setting 15 min after LPS. Vehicle-treated animals served as pathological controls
- Blood was collected 3h or 5h post-LPS challenge and serum was isolated for subsequent analyses. Of note, T_{max} of TZ, the active circulating metabolite of NTZ, was observed in previous PK studies between 3h and 5h. Systemic inflammation was assessed in serum collected at 3 h post-LPS, by quantifying circulating cytokines (IL-6, IL-10, TNF-α, IL-1β and IL-18) using a multiplex sandwich ELISA (Rat Premixed Multi-Analyte Kit LXSARM, Bio-Techne) on a Luminex 200 platform. Hepatic and renal function were evaluated at 5 h post-challenge by measuring serum ALT, total bile acids (TBA), albumin and urea using Randox kits on a Daytona Plus analyzer. Serum cystatin C, as a marker of renal injury, was quantified by ELISA (MSCTC0) with colorimetric detection on a Multiskan Go system

Evaluation of TZ on LPS-induced cytokine release in human PBMCs

- PBMCs were treated for 6 h with tizoxanide (TZ), the active circulating metabolite of nitazoxanide, or rifaximin (both at 0.3 – 30 µM), concomitantly with LPS stimulation (1 ng/mL). Cytokine secretion in culture supernatants was quantified by HTRF assays performed on a Revvity device, using Human IL-6 Detection Kit (Revvity) and TNF-α HTRF kit (PerkinElmer)

Statistical analyses

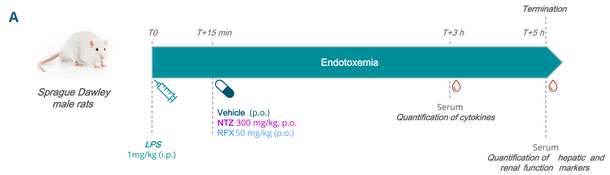
- All statistical analyses were performed using GraphPad Prism version 9.4.0
- For the in vivo study, data were visualized according to their distribution. Parameters following a log-normal distribution were represented as bar graphs with overlaid scatter plots, expressed as geometric mean with 95% confidence intervals (CI). Parameter showing normal distribution (albumin) was presented as bar graph with scatter plots, expressed as arithmetic mean with 95% CI. Between-group comparisons were performed using a Brown-Forsythe and Welch ANOVA followed by false discovery rate (FDR) correction using the original Benjamini-Hochberg method (*p < 0.05, **p < 0.01, ***p < 0.001)
- For the in vitro study, results are presented as dose-response curves, expressed as mean ± standard deviation (SD)

RESULTS

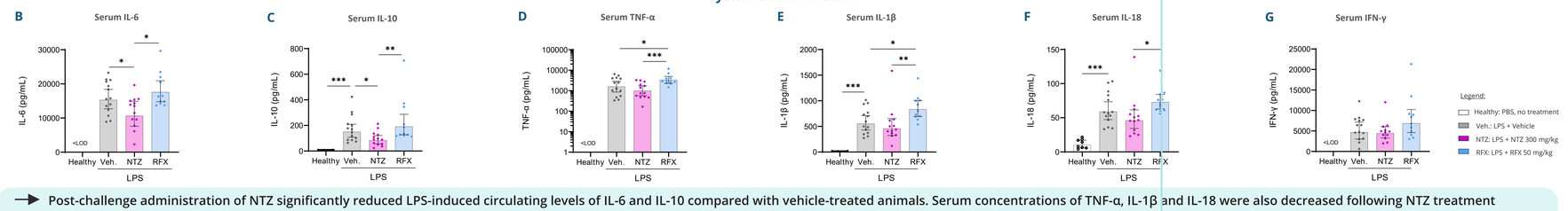
DISTINCT ANTI-INFLAMMATORY AND HEPATOPROTECTIVE EFFECTS OF NTZ VERSUS RFX IN A RAT MODEL OF ENDOTOXEMIA

A. Study scheme
B. Serum IL-6 (T+3 h)
C. Serum IL-10 (T+3 h)
D. Serum TNF-α (T+3 h)
E. Serum IL-1β (T+3 h)
F. Serum IL-18 (T+3 h)
G. Serum IFN-γ (T+3 h)
H. Serum AST (T+5 h)
I. Serum total bile acids (T+5 h)
J. Serum albumin (T+5 h)
K. Serum Urea (T+5 h)
L. Serum cystatin C (T+5 h)

Healthy group: PBS (i.p.), no treatment, n = 8
Vehicle group: LPS (i.p.) + CMC/Tween (p.o.), n = 14
NTZ group: LPS (i.p.) + NTZ 300 mg/kg (p.o.), n = 13
RFX group: LPS (i.p.) + RFX 50 mg/kg (p.o.), n = 11

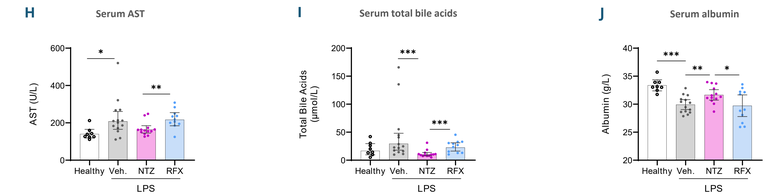


Systemic inflammation



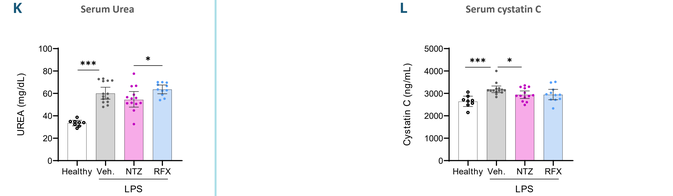
→ Post-challenge administration of NTZ significantly reduced LPS-induced circulating levels of IL-6 and IL-10 compared with vehicle-treated animals. Serum concentrations of TNF-α, IL-1β and IL-18 were also decreased following NTZ treatment
→ Conversely, RFX did not attenuate systemic inflammation and was associated with higher serum cytokine concentrations relative to the vehicle group

Hepatic function



→ NTZ significantly attenuated LPS-induced hepatic dysfunction, as reflected by the decrease in serum levels of AST and total bile acids, together with a restoration of circulating albumin levels, whereas RFX did not improve hepatic function markers in comparison to the vehicle-treated animals

Renal function

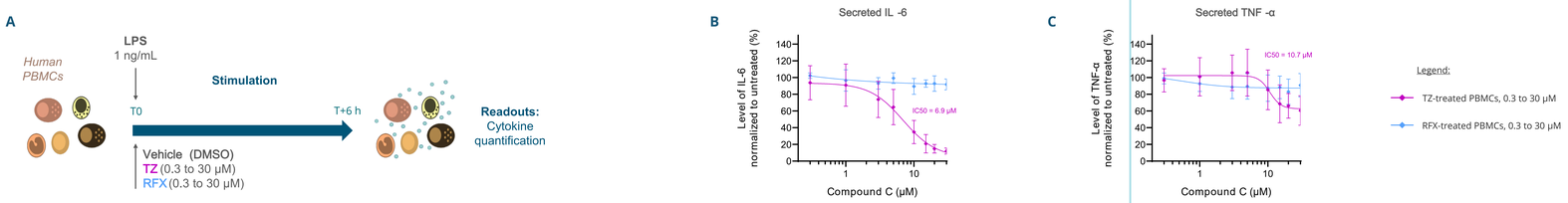


→ NTZ treatment mitigated LPS-induced renal impairment, with a reduction in serum urea and cystatin C levels
→ RFX did not attenuate circulating urea levels, and showed a non-significant decrease in cystatin C levels relative to the vehicle group

DIFFERENTIAL EFFECTS OF TZ AND RFX ON LPS-INDUCED CYTOKINE RELEASE IN HUMAN PBMCS

A. Experimental protocol
B. Secreted cytokine IL-6
C. Secreted cytokine TNF-α

Untreated: LPS + Vehicle, n = 3
TZ: LPS + TZ 0.3 to 30 µM, n = 5
RFX: LPS + RFX 0.3 to 30 µM, n = 6



→ TZ markedly mitigated suppressed inflammatory cytokine secretion in a dose-dependent manner in LPS-stimulated human PBMCs, as evidenced by the decrease in IL-6 and TNF-α
→ In contrast, RFX demonstrated limited inhibitory activity on cytokine release across the tested concentration range

DISCLOSURE

- JT is a consultant for GENFIT SA and a member of EF-CLIFF, which is sponsored by GENFIT. BS is a consultant for GENFIT SA. G1090N (nitazoxanide formulation) is an investigational drug under development for ACLF. In this study, we have used the APIs (Nitazoxanide and Tizoxanide) for in vivo and in vitro experiments
- *We thank Manon Clarisse and Valerie Daix from GENFIT's team for their contribution to this work*

CONCLUSION

- **Although both NTZ and RFX exhibit broad-spectrum antibiotic activity, these results highlight NTZ's superior ability to rapidly mitigate systemic inflammation as well as liver and renal dysfunction in LPS-induced disease models**
- **These findings highlight NTZ (investigational drug G1090N) as a promising pharmacological agent for ACLF and support the interest to further assess the immuno-inflammatory parameters in a phase 2 clinical trial**

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