

EFFECT OF NITAZOXANIDE ON ORGAN FAILURES IN DISEASE MODELS OF ALCOHOL-MEDIATED ACUTE-ON-CHRONIC LIVER FAILURE (ACLF)

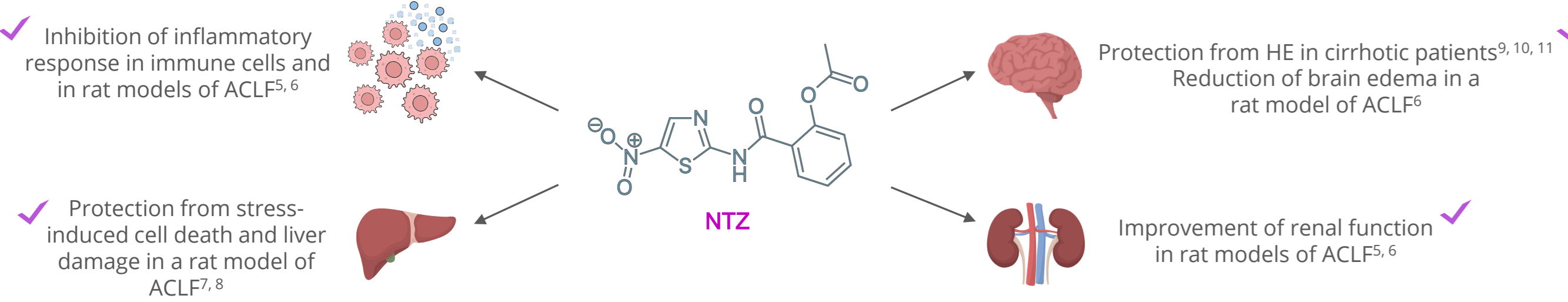
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BACKGROUND & AIM

- 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. Phase 1 clinical trial with G1090N (NCT07110441) confirms favorable safety and tolerability profile
- ACLF is a life-threatening condition marked by organ failures, with short-term mortality increasing with the number of failing organs^{1,2,3}
- Alcohol-related precipitants, particularly severe alcoholic hepatitis, are among the most prevalent triggers of ACLF and drive immune dysregulation and hepatocellular injury⁴
- We previously demonstrated that NTZ alleviates systemic inflammation and organ damage, including liver and kidney dysfunction, in bacterial endotoxin-induced ACLF models^{5,6,7,8}

Evidence of NTZ activity in ACLF:



The efficacy of NTZ and its main circulating active metabolite tizoxanide (TZ) was investigated in alcohol-mediated disease models of ACLF using complementary *in vivo* and *in vitro* approaches:

In vivo study: evaluation of the efficacy of NTZ in a curative setting in an ethanol-induced ACLF rat model with decompensated cirrhosis (ascites), generated by chronic CCl₄ and ethanol exposure and precipitated by an acute ethanol binge (model [CCl₄/EtOH]+EtOH binge), closely recapitulating alcohol-related ACLF observed in patients¹²

In vitro study: assessment of TZ-mediated inhibition of ethanol-induced apoptosis in human hepatocytes

METHODS & STATISTICS

Evaluation of NTZ in an alcohol-mediated ACLF rat model ([CCl₄/EtOH]+EtOH binge)

- Cirrhosis was induced in male Sprague-Dawley rats by chronic CCl₄ administration (dilution 1:1 with corn oil, 1 mL/kg, i.p., twice weekly) combined with ethanol and phenobarbital (0.06 g/L) in drinking water throughout the experiment. Ethanol concentration was progressively increased (4 % v/v during week 1, 8 % v/v during week 2, and 16 % v/v from week 3 until study termination)
- ACLF was precipitated at the decompensated stage by an acute ethanol binge (6 g/kg, p.o.), with decompensation defined by the visual detection of ascites. Animals reached this stage after variable cirrhosis induction periods of around 6-7 weeks (NTZ: n = 8; vehicle: n = 4) or 14-16 weeks (NTZ: n = 1; vehicle: n = 2)
- NTZ was administered orally in a curative setting (150 mg/kg, bid), starting 1 h post-binge and continued for 72 h. Vehicle-treated animals served as pathological controls
- Body weight was measured before termination. Organ failure-related outcomes were assessed prior to sacrifice, including peripheral oxygen saturation (SpO₂) and neurological behaviour. Levels of circulating hepatic and renal marker (blood urea nitrogen, BUN) were quantified in whole blood using Element RCX device (SciLvet). Liver fibrosis was evaluated histologically on paraffin-embedded sections stained with Picrosirius Red. Glutamate and glutamine in cerebellum were quantified using an enzymatic assay based on direct measurement of free glutamate and subsequent glutaminase-mediated conversion of glutamine to glutamate (Glutamine/Glutamate-Glo Assay, Promega). Healthy control animals (n=3) were included retrospectively as a reference group to define baseline cerebellar glutamate and glutamine levels

Evaluation of TZ on ethanol-induced apoptosis in human hepatocytes

- The anti-apoptotic effect of TZ was assessed in human hepatocytes exposed to ethanol (600 mM) under serum-deprived conditions by measuring caspase-3/7 activity and cleaved caspase-3 levels. TZ was added either concomitantly (co-treatment) or 1 h after ethanol stimulation (post-treatment), n = 6 technical replicates per condition
- Caspase-3/7 activity was quantified using a luminescent Caspase-Glo® 3/7 assay (Promega), and cleaved caspase-3 protein levels were assessed by automated capillary-based immunoblotting (Jess system, Protein simple™, using cleaved caspase 3 antibody from Cell Signaling and β-actin antibody from R&D system)

Statistical analyses

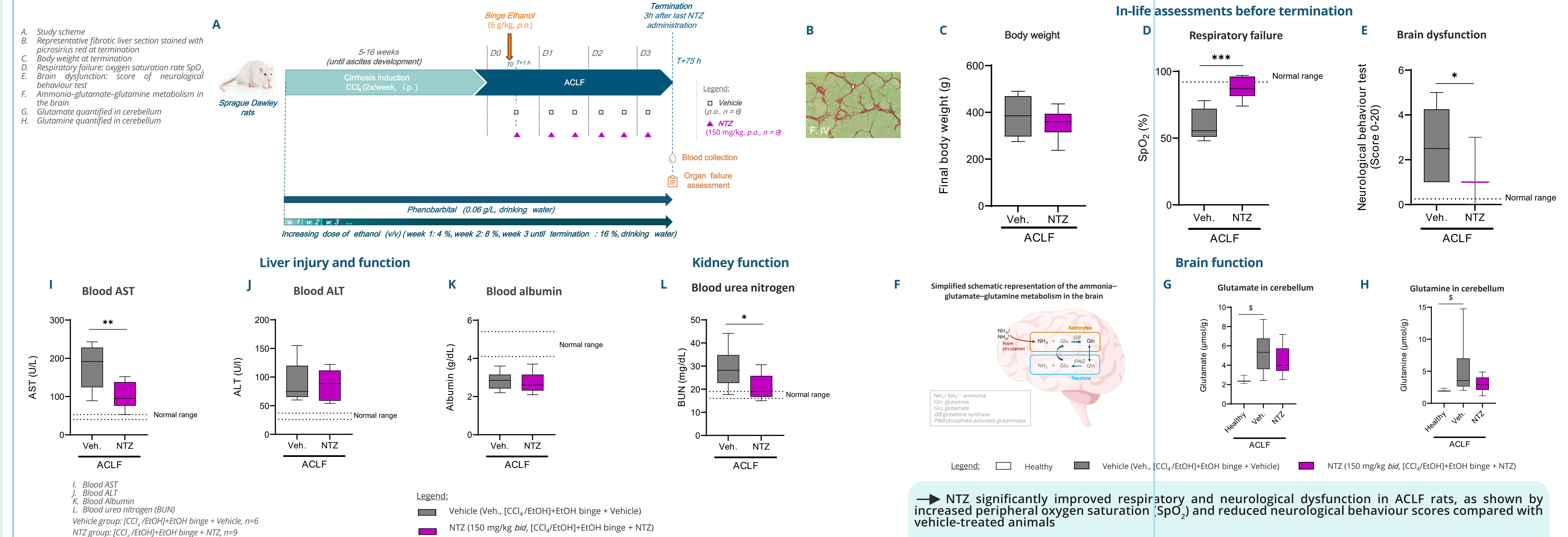
- All statistical analyses were performed on GraphPad Prism version 9.4.0.
- Experimental results are presented as box plots showing the median (line), interquartile range (box: 25th-75th percentile), and minimum to maximum values (whiskers) for *in vivo* study, and as scatter plots showing median (line) and interquartile range (whiskers) for the *in vitro* study
- For normally distributed variables, between-group comparisons were performed using a two-tailed unpaired Student's t-test (* p < 0.05, ** p < 0.01, *** p < 0.001). For non-normally distributed variables, comparisons were conducted using a two-tailed Mann-Whitney test (§ p < 0.05, §§ p < 0.01, §§§ p < 0.001) or using a Kruskal-Wallis test with Dunn's correction for multiple comparisons (§: p<0.05; §§: p<0.01; §§§: p<0.001)

CONCLUSION

- In a disease model of alcohol-mediated ACLF, NTZ was shown to improve multiple organ failures including brain, respiratory system and kidney
- This effect could be in part mediated by a direct effect on cell death, as shown by anti-apoptotic activity *in vitro*
- These findings support NTZ (investigational drug G1090N) as a promising pharmacological agent for ACLF

RESULTS

REPEATED NTZ ADMINISTRATION IMPROVES HEPATIC AND EXTRA-HEPATIC ORGAN FAILURE IN AN ETHANOL BINGE-INDUCED ACLF MODEL

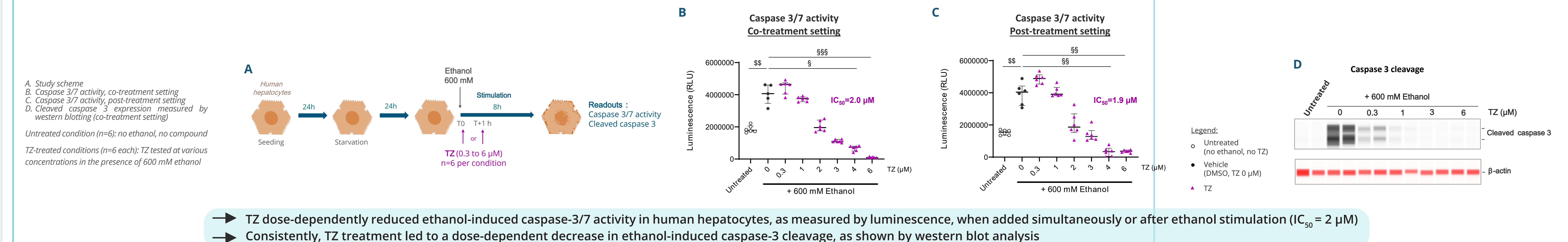


→ Post-binge NTZ treatment significantly improved hepato-renal dysfunction, as shown by marked reductions in AST and BUN, whereas no significant effect of NTZ was observed on ALT and albumin

→ NTZ significantly improved respiratory and neurological dysfunction in ACLF rats, as shown by increased peripheral oxygen saturation (SpO₂) and reduced neurological behaviour scores compared with vehicle-treated animals

→ Alcohol-induced ACLF was associated with elevated cerebellar glutamine, a surrogate of cerebral ammonia detoxification, together with increased glutamate levels, indicating disruption of the glutamate-glutamine cycle. NTZ treatment showed a trend toward reduced levels of both metabolites, consistent with improved neurological behaviour

TZ INHIBITS ETHANOL-INDUCED APOPTOSIS IN HUMAN HEPATOCYTES



- TZ dose-dependently reduced ethanol-induced caspase-3/7 activity in human hepatocytes, as measured by luminescence, when added simultaneously or after ethanol stimulation (IC₅₀ = 2 µM)
- Consistently, TZ treatment led to a dose-dependent decrease in ethanol-induced caspase-3 cleavage, as shown by western blot analysis

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