edema (CE) (29.9% vs. 51.5%) or die to CE by day 21 (4.5%,vs. 11.6%; p < 0.001 for all). Stratifying by TFS status (non-TFS: n = 365 (273 died/100 transplanted) vs. TFS: n = 704), there were no differences in existing psychiatric comorbidity (51.5% vs. 55.0%; p = 0.3) or overdose intention (intentional: 39.7% vs. 41.6%; p = 0.6). During the first 7 days of study, TFS patients were less likely to develop grade 3/4 hepatic encephalopathy (HE; 52.7% vs. 91.5%), required less organ support (mechanical ventilation: 51.3% vs. 90.7%; vasopressors:19.5% vs. 65.8%; CRRT: 13.6% vs. 21.4%), and were less likely to develop cerebral edema by day 21 (CE; 22.1% vs. 61.4%; p < 0.002 for all). On multivariable logistic regression adjusting for vasopressor support (OR 0.25; 95% CI: 0.17–0.35; p < 0.001), development of grade 3/4 HE (OR 0.21; 0.13–0.33; p < 0.001), King's College Criteria (OR 0.53; 0.36–0.78; p = 0.001), and highest MELD score (per unit increase: OR 0.92; 0.90–0.94; p < 0.001), the use of CRRT (OR 1.61; 1.07–2.44; p = 0.02) was associated with TFS (c-statistic 0.86). In a second model substituting enrolment cohort for CRRT, recent enrolment was associated with improved TFS (OR 1.42; 95% CI: 1.03–1.97; p = 0.03; c-statistic 0.86).

Conclusion: Transplant-free survival in APAP ALF has improved over time while the incidence of CE/CE-related death has declined with improved intensive care support possibly related to increased CRRT use.

Cirrhosis – Experimental aspects

AS031

Toll-like receptor 4 inhibition acts synergistically with G-CSF to prevent organ injury and induce liver regeneration in acute-on-chronic liver failure

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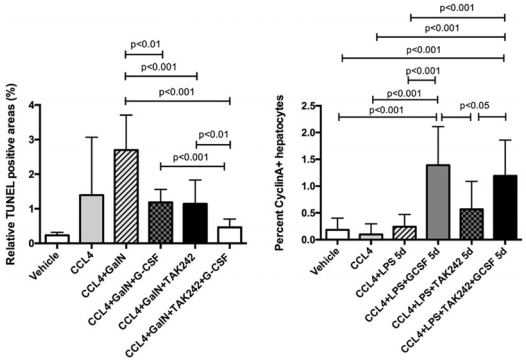
Background and Aims: Acute-on-chronic liver failure (ACLF) is characterised by lack of regeneration. Granulocyte colony stimulating factor (G-CSF) carries pro-regenerative properties and has been shown to be of benefit in ACLF. However, the large trial of G-CSF (GRAFT study) in patients with ACLF showed no benefit and in certain groups mortality tended to be higher. This study was performed to define the mechanisms underlying the negative effect of G-CSF and determine whether its beneficial effect could be harnessed using a toll-like receptor 4 (TLR4) antagonist.

Method: Two mouse models of ACLF were used: CCl4 (0.5 mg/ml,6w) to induce chronic liver injury followed by LPS i.p. (Klebsiella, 4 mg/kg) (n = 4–10) or Galactosamine (GalN) i.p. (1000 mg/kg) as a second hit (n = 8). 1 h after, G-CSF 250 μg/kg s.c. and/or TLR4-inhibitor TAK-242 10 mg/kg i.p. were injected and continued every 24 h. The treatment duration was 24 h and 5 d in the LPS model and 48 h in the GalN model. Samples were stored and analysed for liver injury, inflammation, senescence and regeneration.

Results: 6w CCl4 led to bridging fibrosis, TLR4 up-regulation and infiltration of G-CSFr expressing cells. LPS increased ALT levels, cell death (TUNEL+), enhanced hepatic infiltration of neutrophils (Ly6G+), macrophages (F4/80+) and TNFa. G-CSF increased the 48 h mortality from 0% to 66%, aggravated liver inflammation with macrophage and NK cell (CD45+,CD49b+,CD3-,CD19-) infiltration and IL6 expression. G-CSF+TAK-242 reduced the mortality to 0%, abrogated the liver injury (TUNEL) and liver inflammation (macrophages, neutrophils, TNFa, IL6) significantly. In the second model, GalN also induced a significant liver injury. Treatment with G-CSF+TAK-242 was significantly more effective than the individual therapies (figure). G-CSF +TAK-242 was associated with increased liver regeneration evidenced by increased tissue expression of pSTAT3 and BCL2. CCL4+LPS induced a p53 and p16-dependent cell cycle arrest and lack of proliferation (CyclinA) in hepatocytes. G-CSF+TAK-242 mitigated senescence and significantly increased the rate of CyclinA expressing hepatocytes (figure) suggesting enhanced liver regeneration.



Conclusion: The present study shows that G-CSF is deleterious in LPS-associated ACLF through further activation of inflammatory pathways and immune cell infiltration. TLR4 inhibition with TAK-242 prevented G-CSF driven tissue injury and induced liver regeneration showing evidence of synergy between the two molecules thereby providing a novel therapeutic strategy for ACLF patients.

AS032

A human liver cell-based system modeling a clinical prognostic liver signature combined with single cell RNA-seq for discovery of novel liver disease therapeutics

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