mobility shift assay revealed that DIC attenuated the LPS-induced DNA binding of NF-kB. In addition, it was found that treatment with DIC significantly inhibited the activation of p38 and ERK MAP kinase. Taken together, these results suggest that anti-inflammatory effect of DIC in LPS-treated RAW 264.7 macrophages is associated with the inhibition of NF-kB transcriptional activity, possibly via the MAP kinase pathway. Furthermore, in vivo, DIC conferred increase in the survival of mice that have been subjects to cecal ligation and puncture, an experimental sepsis model.
Conclusions: We believe that the anti-cytokine activity of DIC could be due to the NF-kB/MAPK pathway inhibition. In addition, our data suggest that DIC may potentially play a role in the therapy of inflammatory disease as sepsis.
Keywords: Pro-inflammatory cytokines, Sepsis, 4,5-dihydroisoxasole, NF-kB.
Disclosure of interest: None declared.

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PREVENTIVE EFFECTS OF CHLORELLA ON LPS-INDUCED INFLAMMATION AND SEPTIC DEATH IN MICE

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Inflammation is a host response to infection and is characterized by elevated inflammatory mediators. Two of the major mediators during inflammation, i.e. nitric oxide (NO) and prostaglandin E2 (PGE2), are released by macrophages and neutrophils. Overt inflammation can lead to detrimental consequence, such as septic shock, which is known due to excessive NO and proinflammatory cytokines production. Chlorella has been shown to have various remarkable biological effects, including anti-inflammatory effect through inhibition of iNOS pathway and proinflammatory cytokines (e.g. IL-6 and TNF-α production), however its protection against LPS-induced sepsis has not been studied. In this study, we investigate protective effects of *Chlorella* extract against LPS-induced sepsis in mice. Endotoxin-induced septic shock was initiated by injecting 20 mg/kg of lipopolysaccharide (LPS) in mice. Mice were given different dosages of *Chlorella* extract (10, 20, 30 mg/kg, orally) 30 min after the treatment of LPS. Serum NO production was prepared and measured as nitrite (using Griess reagent), IL-6 and TNF-α were concurrently monitored by using commercial ELISA kits. The mortality rate was observed 12, 24, and 36 h after the LPS treatment.
Chlorella extract administration decreased LPS-induced mortality as evidenced by increased survival rate in a dose dependent manor. The survival rate remained as high as 95% in those mice treated with 30 mg/kg of *Chlorella* extract compared to 40% of survival rate in the control (vesicle treatment) 36 h after LPS injection. *Chlorella* extract treatment also significantly suppressed LPS-induced serum NO, IL-6 and TNF-α levels. In conclusion, *Chlorella* extract effectively prevents LPS-induced septic mortality by reducing inflammatory mediator and proinflammatory cytokines production.
Disclosure of interest: None declared.

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INFLUENZA A VIRUS INDUCES MUC5AC, A MAJOR SECRETED PULMONARY MUCIN, IN A PROTEASE-EGFR-ERK-SP1-DEPENDENT PATHWAY

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Introduction: The airways mucus gel performs a critical function in the defense of the respiratory tract against pathogenic and environmental challenges. In respiratory infections, mucins such as Muc5AC could be protective and able to sequester pathogens [1].
Aims: (1) To determine whether IAV (seasonal and pandemic Influenza A strains) is able to modulate Muc5AC production in airway epithelial cells. (2) To dissect the molecular pathways involved in IAV modulation of Muc5AC synthesis.
Results: We demonstrate here that seasonal and pandemic strains infection of NCI-H292 cells (MOI = 1) induced significantly MUC5AC gene expression. Influenza A/Scotland/20/74 (H3N2), the most virulent strain, lead to a significant Muc5AC increase compared to mock-treated cells, and this was dependent on viral replication. Moreover, using the same strain, we showed that in vivo infection of C57Bl/6 mice with 150 or 300 pfu lead to a threefold increase of Muc5ac RNA expression at day 3 and 4 post-infection compared to PBS-treated mice. We demonstrated that the Sp1 transcription factor is involved in IAV-induced MUC5AC gene expression and that this is mediated by the MEK/ERK signaling pathway. Next, we showed that this pathway was initiated upstream by a matrix metalloproteinase (TACE)-mediated epidermal growth factor receptor (EGFR) activation and that TGF-a is one of the ligands implicated in EGFR activation [2, 3].
Conclusion: We show for the first time that IAV up-regulates Muc5AC through a TACE/EGFR/MAPK pathway and that this induction is IAV-replication dependent. We are studying now if up-regulation protects against IAV infection or whether Muc5AC over-expression is deleterious in IAV-induced deleterious exacerbations in chronic lung diseases, such as asthma or cystic fibrosis.

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