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Epigallocatechin gallate treatment decreases osteopontin expression and attenuates N-Nitrosodimethylamine induced hepatic fibrosis in rats

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Background and Aims: Osteopontin (OPN) is a matricellular cytokine and a stress-induced pro-fibrogenic molecule that promotes activation of stellate cells during pathogenesis of hepatic fibrosis. The current investigation was aimed to study the effect of epigallocatechin gallate (EGCG) to decrease osteopontin expression and subsequent arrest of experimentally induced hepatic fibrosis. Methods: The liver injury was induced with intraperitoneal injections of N-nitrosodimethylamine (NDMA) in a dose of 1 mg/100 g body weight on 3 consecutive days of every week for 4 weeks. Another group of animals received 0.2 mg EGCG/100 g body weight orally 2 h prior to NDMA administration. The animals were sacrificed at the end of 2nd week and 4th week from the beginning of exposure. Serum OPN, type IV collagen, and hyaluronic acid (HA) levels were measured. Glutathione and malondialdehyde levels were determined in the fresh liver tissue. The paraffin liver sections were stained for collagen, α-SMA, 4-HNE and OPN. Western blotting and qPCR were performed for OPN. Results: Serum OPN levels were increased in both early and advanced fibrosis and the data were significantly correlated with collagen, α-SMA, 4-HNE, and OPN staining in the liver. EGCG treated animals depicted a significant decrease of OPN, type IV collagen, and HA in the serum as well as staining for collagen, α-SMA, 4-HNE, and OPN in the liver. Western blotting and qPCR for OPN demonstrated marked reduction in OPN expression. Furthermore, animals treated with EGCG maintained antioxidant status and exhibited marked decrease of hepatic fibrosis. Conclusions: The data demonstrated that serum OPN correlates with the degree of hepatic fibrosis. Treatment with EGCG resulted in decrease of oxidative stress and reactive oxygen species and prevented upregulation of OPN with a subsequent decrease of hepatic fibrosis. EGCG could protect against chronic liver injury and fibrogenesis and could pave the way for therapeutic intervention of hepatic fibrosis.

Disclosures:

The following people have nothing to disclose: Joseph George, Mikihiro Tsutsumi

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Iron excess exacerbates proinflammatory and fibrogenic gene expression in genetically obese mice and in primary human hepatic stellate cells in response to TNF-α or TGF-β

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Aim: We have previously shown that reticuloendothelial system (RES) iron is associated with increased severity of nonalcoholic steatohepatitis (NASH) and advanced fibrosis in nonalcoholic fatty liver diseases. We hypothesized that an acute iron overload in obese (Leprdb/db) mice or iron loading of primary human hepatic stellate cells would cause inflammatory NF-KB and profibrogenic activation. Further, we asked if iron overload could exacerbate the inflammatory/fibrogenic effect of cytokines such as TNF-α or TGF-β in the primary human stellate cells. Methods: Obese leptin receptor deficient (Leprdb/db) were maintained on normal chow for 8 weeks before receiving a single dose of 1.25 mg/g wt Fe-dextran by IP injection. They were fed a normal chow or a high fat (HF) diet for 8 weeks after IP injection. After 16 weeks, the chow - fed (C), the PI (parenteral iron) mice, PI+ HF mice, and the HF-fed mice were sacrificed, and their livers were assessed for hydroxyproline (a measure of collagen content) and changes in gene expression related to fibrogenesis. To determine if iron overload had an effect on primary human stellate cells, a time course study was performed with ferric ammonium citrate (FAC) between 0-6 hours and gene expression changes related to fibrogenesis and NF-KB activation were measured. Results: PI+HF mice livers had significantly higher levels of hydroxyproline relative to chow-fed controls. PI mice showed high levels of ASMA gene expression relative to controls. Parenteral iron administration also led to elevated levels of NF-KB-dependent proinflammatory genes such as TLR4, MCP-1 and iNOS. In the primary human stellate cells, we found that increasing exposure time to iron showed an elevation in gene expression of NF-KB, and NF-KB-dependent genes such as MCP-1, TNF-α, IL-6, IL-1β and HO-1, with 4-6 hours yielding the highest expression. We also found that collagen1α1, MMP-2, TIMP-1 and TGF-β were upregulated in response to iron. We asked if iron in the presence of cytokines such as TNF-α or TGF-β would potentiate proinflammatory and profibrogenic gene expression and found that coadministration of iron/TNF-α led to synergistic upregulation of TNF-α, IL-6 and collagen1α1 levels; and cotreatment with iron/TGF-β led to heightened expression of TIMP-1, TGF-β and collagen1α1. Conclusions: In a mouse model of genetic obesity, acute iron excess coupled with a HF diet promotes fibrogenesis. In primary human stellate cells, iron overload, either alone or in conjunction with cytokines, elevates proinflammatory and fibrogenic gene activation. These findings highlight a key role for iron as a profibrotic agent in accelerating NASH progression.

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Modulating Tumor-Stromal Interactions by Targeting Adenosine Monophosphate-Activated Kinase (AMPK) in Human Hepatic Stellate Cells and Hepatocellular Carcinoma

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AIM: End stage liver disease is characterized by liver fibrosis and cirrhosis, a major risk factor for the development of hepatocellular carcinoma (HCC). Human hepatic stellate cells (hHSC) are the key players in liver fibrosis associated with HCC. Hence, tumor-stromal interactions are considered a potential target for anticancer and antifibrogenic therapies. A key regulator of both hHSC and HCC is adenosine monophosphate-activated kinase (AMPK), a fuel-sensing enzyme. AMPK has been implicated in carcinogenesis as liver kinase B1 (LKB1), a tumor suppressor, is an upstream activating kinase for AMPK. The LKB1-AMPK axis has been shown to suppress cell proliferation via the mTOR signalling pathway in solid tumors. However, recent data demonstrate an LKB1-independent tumor suppressing effect of AMPK, indicating a distinct role for AMPK in cancer development. In this study, the bi-di-