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Apigenin inhibits pancreatic stellate cell activity in pancreatitis

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ABSTRACT

Background: Chronic pancreatitis (CP) is characterized by recurrent pancreatic injury, resulting in inflammation, necrosis, and fibrosis. There are currently no drugs limiting pancreatic fibrosis associated with CP, and there is a definite need to fill this void in patient care.

Materials and methods: Pancreatitis was induced in C57/BL6 mice using supraphysiologic doses of cerulein, and apigenin treatment (once daily, 50 μg per mouse by oral gavage) was initiated 1 wk into the recurrent acute pancreatitis (RAP) protocol. Pancreata were harvested after 4 wk of RAP. Immunostaining with fibronectin antibody was used to quantify the extent of pancreatic fibrosis. To assess how apigenin may decrease organ fibrosis, we evaluated the effect of apigenin on the proliferation and apoptosis of human pancreatic stellate cells (PSCs) *in vitro*. Finally, we assessed apigenin's effect on the gene expression in PSCs stimulated with parathyroid hormone–related protein, a profibrotic and proinflammatory mediator of pancreatitis, using reverse transcription-polymerase chain reaction.

Results: After 4 wk of RAP, apigenin significantly reduced the fibrotic response to injury while preserving acinar units. Apigenin inhibited viability and induced apoptosis of PSCs in a time- and dose-dependent manner. Finally, apigenin reduced parathyroid hormone–related protein–stimulated increases in the PSC messenger RNA expression levels of extracellular matrix proteins collagen 1A1 and fibronectin, proliferating cell nuclear antigen, transforming growth factor-beta, and interleukin-6.

Conclusions: These *in vivo* and *in vitro* studies provide novel insights regarding apigenin's mechanism(s) of action in reducing the severity of RAP. Additional preclinical testing of apigenin analogs is warranted to develop a therapeutic agent for patients at risk for CP.

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1. Introduction

Chronic pancreatitis (CP) is a progressive, irreversible disease process characterized by chronic inflammation, glandular necrosis, and fibrosis [1]. With repeated injury, functional pancreatic tissue is replaced with a fibrotic scar; and when pancreatic reserve is exhausted, exocrine and endocrine insufficiencies develop [2]. Patients have a poor quality of life and are burdened by chronic abdominal pain, impaired digestion, malnutrition, anorexia, diabetes, and disease-related complications such as pseudocyst formation [3]. CP also increases a patient's risk of developing pancreatic cancer [4].

Accumulating genetic, clinical, and experimental evidence support the hypothesis that CP is the result of multiple episodes of recurrent acute pancreatitis (RAP) [5–7]. The risk factors that are associated with the development of CP include alcohol consumption, smoking, nutritional factors, hereditary predisposition, efferent duct obstruction, immunologic factors, and metabolic disease [2]. Irrespective of the etiology, pancreatitis involves a common cascade of events as follows: acinar cell injury causing aberrant zymogen secretion and premature activation, tissue autodigestion, generation of an inflammatory response, focal necrosis, and fibrosis [5,6,8]. With recurrent episodes of acute pancreatitis (AP), the pancreas is unable to adequately recover from the repeated injury, perpetuating a microenvironment of chronic inflammation and irreversible fibrosis [5,6].

Pancreatic stellate cells (PSCs) are responsible for generating the characteristic glandular scarring of CP [9]. Pancreatic injury promotes the activation of PSCs, which rapidly proliferate, migrate to sites of injury, synthesize and remodel extracellular matrix (ECM) proteins, and secrete cytokines and growth factors, further amplifying the immune response [9,10]. Parathyroid hormone–related protein (PTHrP) has been identified as a profibrotic and proinflammatory mediator of AP and CP [11,12]. PSCs not only express the G protein–coupled receptor for PTHrP but also have been shown to secrete the PTHrP protein in response to injury [11–14].

Currently, treatment options for CP are limited to supportive care and symptom palliation. Patients must adapt to a lifestyle involving chronic pain management, digestive enzyme replacement, vitamin supplementation, and glucose control [3]. Medical management often fails with advanced disease, and patients are offered more invasive interventions, ranging from endoscopic stenting of strictures to surgical bypass procedures or even total pancreatectomy [15]. There is a definite need to develop pharmacologic agents directed at the pathogenesis of CP, reducing pancreatic damage, inflammation, and fibrosis.

Apigenin (4′,5,7-trihydroxyflavone) is a natural compound with low intrinsic toxicity, found in various fruits, vegetables, herbs, and beverages such as chamomile tea [16]. Herein, we report how apigenin protects the pancreas from repeated pancreatic injury, and therefore slows the development of CP. This is accomplished, in part, by apigenin as follows: 1) inhibiting PSC proliferation; 2) inducing PSC apoptosis; and 3) and minimizing PTHrP-mediated PSC response to injury. Our data suggest that apigenin and/or apigenin-like compounds could be developed into novel pharmacologic inhibitors of RAP progression in patients at risk for CP.

2. Materials and methods

2.1. Materials

Cerulein (CR) was purchased from Bachem (Torrance, CA). Apigenin was purchased from Sigma–Aldrich (St. Louis, MO). The human parathyroid-related protein (1-36) was purchased from PolyPeptide Laboratories (San Diego, CA). The following reagents were purchased from DAKO (Carpinteria, CA): target retrieval solution, antibody diluent, liquid 3,3'-diaminobenzidine (DAB), and substrate chromogen system. Fibronectin antibody was purchased from Santa Cruz Biotechnology (Dallas, TX). The following products were purchased from Vector Laboratories, Inc (Burlingame, CA): biotinylated secondary antibody, VECTASTAIN Elite ABC kit, and VectaMount. The hematoxylin 7211 counterstain was purchased from Thermo Fisher Scientific, Inc (Kalamazoo, MI). Cell culture reagents were used from the following companies: Dulbecco's Modified Eagle Medium (DMEM) (VWR, Radnor, PA); collagen from calf skin, penicillin, streptomycin, amphotericin, and gentamicin (Invitrogen, Carlsbad, CA); insulin-transferrin-selenium-ethanolamine (Gibco, Grand Island, NY); nonessential amino acids (Sigma–Aldrich); and fetal bovine serum (FBS; Lonza, Walkersville, MD).

2.2. RAP model of chronic pancreatitis

All animal studies were approved by the University of Texas Medical Branch Institutional Animal Care and Use Committee, which is fully accredited by the Association for Assessment and Accreditation of Laboratory Animal Care International. We used a well-established model of recurrent pancreatic injury to induce CP. Repeated administration of the cholecystokinin analog, CR, leads to hyperstimulation of pancreatic acinar cells, aberrant zymogen secretion, and premature activation [17,18]. Over time, the repeated cycles of injury and inflammation result in the histologic and pathophysiological characteristics of CP [8,17]. Male and female C57/BL6 mice (Harlan Laboratories, Indianapolis, IN; and The Jackson

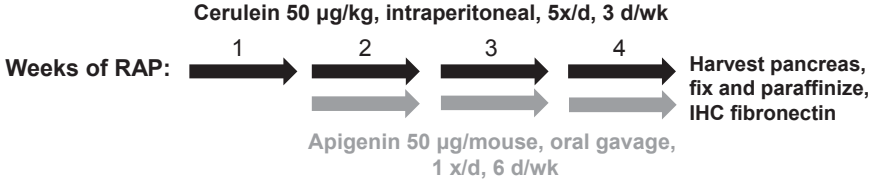

Fig. 1 – Schematic of translational RAP model in mice. IHC = immunohistochemistry.