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Novel Antioxidant Ameliorates the Fibrosis and Inflammation of Cerulein-Induced Chronic Pancreatitis in a Mouse Model

Byung-Moo Yoo[a] Tae-Young Oh[a] Young-Bae Kim[a] Marie Yeo[a]
Jeong-Sang Lee[b] Young Joon Surh[b] Byoung-Ok Ahn[a] Wook-Hwan Kim[a]
Seonghyang Sohn[a] Jin-Hong Kim[a] Ki-Baik Hahm[a]

[a]Genome Research Center for Gastroenterology and Department of Gastroenterology, Ajou University School of Medicine, Suwon, and [b]College of Pharmacology, Seoul National University, Seoul, Korea

Key Words
Chronic pancreatitis · Fibrosis · Mice model · Cerulein-induced pancreatitis · Pancreatic stellate cell · Oxidative stress · DA-9601

Abstract
Background/Aim: Oxygen free radicals (OFRs) mediate an important step in the initiation of experimental acute pancreatitis and several clinical findings suggested the possible contribution of OFRs to the pathogenesis of pancreatic fibrosis. So far, there are no studies which reporting potential role of OFRs in development of chronic pancreatitis with the prevention with antioxidants. This study was aimed to establish the mice model of chronic fibrosing pancreatitis and to prove the involvement of OFRs in chronic pancreatitis with fibrosis. ***Methods:*** Repeated intraperitoneal cerulein injection was performed to induce chronic pancreatitis in mice. Histological changes in the pancreas were examined, and markers for oxidative stress were measured in the pancreatic tissue and serum of the mice. DA-9601, a phytochemical possessing anti-inflammatory and antioxidative action, was given together with cerulein to the mice. ***Results:*** Repeated intraperitoneal injection of cerulein provoked significant severity of chronic fibrosing pancreatitis after 5 weeks. After treatment of DA-9601, the extents of pancreatic fibrosis were statistically significantly decreased in accordance with lessened pancreatic inflammations. The NF-κB binding activities were increased in chronic pancreatitis, which were significantly attenuated after DA-9601 treatment. The levels of myeloperoxidase and iNOS activities were also significantly decreased in DA-9601-treated group compared to the pancreatitis only group. Cytoprotective proteins such as heat shock protein-70 (HSP) and metallothionein were significantly increased in the DA-9601-treated group. DA-9601 decreased the expressions of α-SMA and type I collagen in cultured pancreatic stellate cells. ***Conclusions:*** Oxidative stress was principally involved in the pathogenesis of chronic pancreatitis with fibrosis.

Introduction

Chronic pancreatitis is characterized histologically by an irreversible and irregular scarring of exocrine parenchyma with ductal changes by inflammatory processes [1] and morphologically it is also defined as progressive pan-

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Prof. Ki-Baik Hahm
Department of Gastroenterology, Ajou University School of Medicine
San 5 Wonchon-dong, Paldal-ku
Suwon 442-749 (Korea)
Tel. +82 31 219 4381, Fax +82 31 219 4399, E-Mail hahmkb@hotmail.com

creatic fibrosis and atrophy of the pancreatic exocrine cell mass [2]. The mechanisms by which these fibrotic processes occur are not fully understood. It has been shown that extracellular matrix (ECM) components are distributed in tissue samples of both healthy and diseased pancreas, suggesting an increased deposition of disorganized matrix components in chronic pancreatitis [3, 4].

Studies on the pathogenic mechanisms of fibrosis in human chronic pancreatitis are restricted by limited availability of tissues obtained from surgery. Therefore, animal models have been used to investigate the initiation and promotion of chronic pancreatitis. Although there are numerous well-defined models of acute pancreatitis [5–7], reproducible models showing irreversible pancreatic fibrosis are lacking since experimental models dealing with introduction of fibrosis are time-consuming and not so successful [8]. However, animal models are indispensably required for documenting the underlying mechanism of fibrogenic process and documenting pathophysiology of chronic pancreatitis [9].

Under the hypothesis that repetitive and self-limited acute acinar cell injury with a short interval between injuries could cause the morphologic changes of chronic pancreatitis in experimental animals, several researchers have tried to generate the model of chronic pancreatitis in diverse animals, but such tries could only be possible in a few mice models [10–12]. Until now, some of those models could serve as a reliable tool for identifying the critical signaling events that lead from repetitive acute injury to the accumulation of excess ECM and loss of acinar cell function [10, 12], but none showed definite evidence that some interventions could be tried for the search of either novel treatment modality or concrete pathogenic process.

Oxygen free radicals (OFRs) mediate an important step in the initiation of experimental acute pancreatitis [13]. Although the first to study a possible involvement of OFR were using an ex vivo canine pancreas preparation [14], currently cerulein-induced pancreatitis model is used most commonly because of simplicity and high reproducibility and it was known that the histological changes of pancreas in such animal model were mediated by OFRs [6]. However, due to the lack of adequate experimental animal models for chronic pancreatitis, the involvement of OFRs in chronic inflammation of the pancreatic gland has not yet been studied.

Several clinical findings such as increased lipid peroxidation markers in the duodenal juice and serum of patients with chronic pancreatitis and increased conjugated diene concentrations in pancreatic tissues suggested a major contribution of oxygen free radicals in the pathogenesis of chronic pancreatitis [7]. So far, there have been no studies on the potential effects of oxidative stress and free radicals in animal model of chronic pancreatitis.

The aim of this study was to establish the mice model of chronic pancreatitis as well as to prove the involvement of OFRs in chronic pancreatitis to show the usefulness of antioxidant, DA-9601 in the current experiment, in the treatment of chronic pancreatitis. DA-9601, a novel antioxidative phytochemical derived from the ethanol extract of *Artemisia asiatica*, a remedy used for a long time in folk medicine for several inflammatory diseases, was administered. According to our study group [39, 40], this substance was found to possess antioxidative, anti-inflammatory, and cytoprotective actions.

Material and Methods

Animals and Experimental Procedures

Eight-week-old male ICR mice (Charles River Japan) weighing 20–22 g were housed in isolated cages (4 mice/cage) and fed rodent chow pellets (Purina Korea, Korea) ad libitum. Following a 5-day equilibration period after delivery from the supplier, mice were treated with cerulein (Sigma, St. Louis, Mo., USA), 40 μg/kg intraperitoneally every hour for 6 h to induce acute pancreatitis. The abdominal fur was swabbed with an antiseptic before injections. Induction of acute pancreatitis was repeated every Monday and Thursday for 5 or 10 weeks. At predetermined time points after the first treatment, animals were sacrificed at 4 days following the final cerulein treatment by cervical dislocation. A total of 75 mice were included in the current experiment, and were divided largely into the following two groups; the first group (total 30 mice) was sacrificed at 5 weeks after the initial injection of cerulein (total 10 episodes of acute pancreatitis), and the other group (total 30 mice) was killed after 20 episodes of cerulein injections. Half of the mice in each group were administered pellet diets containing DA-9601 (100 mg/kg), and the remaining half were administered conventional pellet diets. The amounts of pellet diets consumed in both groups were monitored continuously through the entire experimental periods. For the age-matched non-treated normal control, 15 mice were maintained all through the experiment in an isolated room (fig. 1).

Histological Evaluation with Scoring System

For light-microscopic examination, pancreatic specimens were fixed overnight in 10% buffered formaldehyde solutions, embedded in paraffin, and stained with hematoxylin-eosin and Masson's trichrome. Based on the criteria of histological scoring, 0 = none, 1 = focal periductular fibrosis, 2 = diffuse periductular fibrosis, 3 = focal acinar atrophy, 4 = diffuse acinar atrophy, semiquantitative scoring was performed by two independent pathologists in a blind fashion using pancreatic preparations from animals of each group. Figure 2 showed the photographs containing the criteria of each score. In each mouse pancreas, the pancreatitis and fibrosis was not homogenous. Therefore, we adopted a scoring system and made the sum of five different sites in each mouse and represented as the mean score per mouse with the presented scoring system in figure 2.