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The role of apigenin in an experimental model of acute pancreatitis

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ABSTRACT

Aim: The aim of the present study is to evaluate pathologic changes in the pancreatic parenchyma in an experimental model of acute pancreatitis (AP) following bilio-pancreatic duct ligation. An effort was made to clarify the role of apigenin, a substance that is well-known for its antioxidant and anti-inflammatory role and its likely beneficial activity to the pancreatic parenchyma following AP in rats.

Material and method: One hundred twenty-six male Wistar rats 3–4 mo old and weighing 220–350 g were used. At time 0, the following groups were randomly assigned: group sham: rats were subjected to virtual surgery; group control: rats were subjected to surgery for induction of AP, by ligation of the bilio-pancreatic duct; group apigenin: rats were subjected to surgery for induction of AP and enteral feeding with apigenin. Pathologic changes of the pancreatic parenchymal and myeloperoxidase activity were measured at predetermined time intervals 6, 12, 24, 48, and 72 h.

Result: From the pathologic reports, by comparing the control group with the apigenin group, an improvement of pancreatic tissue architecture following apigenin administration was observed. Inflammatory infiltration, edema, ductal dilation, and necrosis were reduced following apigenin administration over time ($P = 0.049$, $P = 0.228$, $P = 0.387$, $P = 0.046$). Treatment with apigenin significantly reduced the bilio-pancreatic duct ligation and evoked an increase in pancreatic myeloperoxidase activity ($P = 0.030$).

Conclusion: Oral apigenin administration in rats, following experimentally induced pancreatitis, seems to protect the pancreatic tissue. Thus, apigenin administration to humans could potentially ameliorate the damages to the pancreas.

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1. Introduction

Acute pancreatitis (AP) is a quite common inflammatory disease which in 10%–20% of all cases leads to severe AP [1], involving remote organ failure and finally multiple organ dysfunction syndrome. The precise mechanisms that initiate an episode of AP are not clearly understood but once initiated; all cases share the same inflammatory and repair pathways [2]. Acute lung injury is almost always present in cases of severe AP [3] and manifests itself as acute respiratory distress syndrome [4], as a result of the systemic inflammatory response syndrome. The most important inflammatory mediators involved in the pathogenesis of AP are tumor necrosis factor-alpha, interleukin (IL)-1β, IL-6, platelet activating factor, IL-10, granulocyte macrophage-colony stimulating factor, C5a, intercellular adhesion molecule-1, reactive oxygen species, and reactive nitrogen species [5–7].

Mortality rates of AP are related to necrosis, bacterial contamination, and pancreatic ascites. Thus, in cases of pancreatic necrosis, the mortality rate ranges from 7% if the necrosis is <30% of the pancreatic tissue to 50% if more than 50% of the pancreatic tissue is necrotized. Bacterial contamination triples the mortality rate from 10% to 32%, whereas the presence of pancreatic ascites increases the rate from 9% to 36% [8].

Bearing in mind that inflammatory mediators play a crucial role in the progression of pancreatitis from mild edema of the pancreas to multiple organ dysfunction syndrome, there is an ongoing research to find evidence-based pharmacological treatment focused on targeted anti-inflammatory drugs [9,10]. Apigenin (4,5,7 trihydroxyflavone) found in fruits, plants, and vegetables is known for its anti-inflammatory, antioxidant, anti-allergic, anti-osteoporotic, and even anti-cancerous activities [11–14]. To the best of our knowledge, apigenin has never been studied before as a possible therapeutic agent in experimental models of AP.

Early events of AP in humans are not possible to be studied in depth for two main reasons. First, the diagnosis of AP is usually set late, once damage to the pancreas has been initiated, and second, there is no available biopsy material from patients with early onset AP. This fact has made efforts to find an ideal experimental model of AP popular. There are a significant number of such models developed, each with its own advantages and drawbacks [15–18]. Duct obstruction induced pancreatitis model can mimic both benign and malignant disorders of the humans [18]. This model does not require special surgical dexterity and once ligation occurs close to the entry of the common bilio-pancreatic duct to the duodenum, the model resembles gallstone obstruction at the ampulla of Vater [18–20].

The purpose of the present study is to evaluate the pathologic changes to the pancreatic parenchyma in an experimental model of AP in rats, following ligation of the bilio-pancreatic duct close to the duodenum and to examine the possible beneficial role of apigenin. In order to accomplish this, myeloperoxidase (MPO) activity of the pancreatic tissue will be examined, along with all histopathologic parameters of AP.

2. Materials and methods

2.1. Animals and design of the study

One hundred twenty-six Wistar male rats, 3–4 mo old and weighing 220–350 g, were used in this study. All rats were maintained under conventional conditions of controlled temperature (22–25°C), humidity (55%–58%), and lighting (12 h light/12 h dark), with free access to tap water and rat chow diet. The animals were supplied by Pasteur Institute, Athens, Greece; all experiments took place at the approved Experimental Research Center of ELPEN Pharmaceuticals, Athens, Greece, while the pathology examination took place at the Laboratories of the Medical School of Democritus University of Thrace. The experimental procedures conform to National Research Council Guide for the Care and Use of Laboratory Animals and Directive 86/609 of the European Union, protocol number (K/2284). The experimental animals were randomly assigned in three groups, namely sham group ($n = 20$), control group (induction of pancreatitis) ($n = 56$), apigenin group (induction of pancreatitis plus administration of apigenin) ($n = 50$). This unbalanced randomization design does not compromise the statistical power [21,22].

2.2. Experimentally induced AP in rats

Before surgery rats were anesthetized in a specially designed glass box for about 2 to 3 min with isoflurane, following a subcutaneous injection of 0.25 cc of butorphenol (Dolorex; Intervet/Schering/Plough Animal Health, Boxmeer, Holland). Soon thereafter, endotracheal intubation was performed under direct laryngoscopy by a trained veterinarian and research assistants with the use of a 16-G venous catheter connected to a rodent ventilator (Harvard Apparatus, Holliston, MA) at the following settings: tidal volume: 3 mL; rate: 70 breaths/min. Proper intubation was confirmed by observation of chest expansion and retraction and lung auscultation. Anesthesia maintenance was accomplished using a mixture of 93% O_2, 5% CO_2, and 2% isoflurane (Fig. 1A and B).

Acute pancreatitis was induced as described previously [18], by ligation of the common bilio-pancreatic duct close to the duodenum. Briefly, soon after anesthesia, a 3 cm midline incision was performed under sterile conditions and entry to the abdominal cavity was accomplished. The bilio-pancreatic duct was identified and ligated close to the duodenum with a 4-0 silk suture (Fig. 1C). Before closure of the abdominal cavity with vicryl 2-0, 1 cc of natural saline and 1 cc D_5W were instilled. All animals regained consciousness as soon as they were extubated. For the sham operated animal group, the experiments were terminated without the ligation step. Soon after the midline incision, the intraperitoneal pancreas was identified, manually mobilized, and no further action was taken before closure of the abdominal cavity. For the apigenin animal group, after termination of the surgical ligation procedure, a prepared 4 cc apigenin solution was administered orally, as described later (Fig. 1D). Analgesia (2 cc/kg butorphenol, Dolorex) for all animals was given subcutaneously at a predetermined time every 4 h, subsequently according to the animal's need based on the clinical picture.