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Pirfenidone prevents rat esophageal stricture formation

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ABSTRACT

Background: Accidental ingestion of caustic substances induces esophageal injuries and stenosis formation. The main aim for acute phase treatment is to prevent esophageal stenosis. Pirfenidone (PFD) is a pyridone with antifibrotic and anti-inflammatory effects. Esophagus stenosis takes place after a strong inflammation process where proinflammatory and profibrogenic cytokines play an important role. The present study investigates the efficacy of PFD on the prevention of stricture development after esophageal caustic injuries in a rat model.

Material and methods: Caustic esophageal burn was produced by application of 32% of NaOH to the distal esophagus of healthy rats. PFD in the form of 8% gel was administered at a dose of 200 mg/kg/d. Animals were divided in three experimental groups as follows: healthy rats, animals injured with NaOH without PFD treatment, and rats injured with NaOH and treated with PFD. Efficacy of the treatment was assessed by measuring image esophagoscopy and esophagography with contrast barium at the 21st d. Histology staining with Sirius-red was performed to evaluate collagen deposition and stenosis area. Gene expression of transforming growth factor β1, collagen-1, plasminogen activator inhibitor-1, connective tissue growth factor, and matrix metalloproteinase 2 were measured by quantitative reverse transcription polymerase chain reaction.

Results: There was significant difference in means of stenosis by esophagoscopy and esophagogram. Collagen deposition in the damaged area increased significantly when rats were burned with NaOH, and decreased notably in PFD treated group. Profibrogenic key molecules transforming growth factor β1, collagen 1, plasminogen activator inhibitor-1 and connective tissue growth factor expression were significantly lower respect to control group without PFD treatment where matrix metalloproteinase 2 expression was no different in all groups.

Conclusions: This study suggests that PFD reduces stenosis on caustic esophageal burn by decreasing profibrogenic genes expression and ameliorates fibrosis significantly in the chronic phase.

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1. Introduction

Accidental ingestion of caustic substances induces esophageal injuries and stenosis formation. Children aged ≤5 y are especially vulnerable for accidental ingestion of toxic substances and represent a significant problem [1]. In Mexico, lack of socially oriented programs to prevent this type of household accidents, along with opportune and timely medical attention to minimize gastrointestinal damage, worsens the clinical outcome [2].

The main aim for acute phase treatment of caustic esophageal burn injury is to prevent stricture formation. Stricture onset or esophagus stenosis will take place after the inflammatory phase where proinflammatory and profibrogenic cytokines perform their deleterious function. Although several treatment protocols have been devised and used to accomplish anti-inflammatory and antifibrogenic actions, the benefit and standardization of these treatment methods are still controversial [3]. Nowadays, the effects of many therapeutic agents in preventing esophageal stricture formation continue to be investigated in experimental caustic esophageal burn injury models [4,5].

Pirfenidone (PFD) is an orally available pyridone derivative that has antifibrotic and anti-inflammatory effects. Since its discovery as an antifibrotic agent in a hamster model of bleomycin-induced pulmonary fibrosis [6], PFD has been tested in a variety of cellular and animal models of inflammation and fibrosis and has been shown to have anti-inflammatory, antioxidant, and antiproliferative properties [7,8]. Beneficial effects have been shown for PFD in the treatment of fibrotic diseases, including renal, liver, and pulmonary fibrosis, multiple sclerosis, and hypertrophic scars conditions. All these diseases share most of the cellular and molecular mechanisms involved in the pathology of abnormal deposition of collagen, which is determinant for a poor clinical outcome [9]. In these fibrosis-related diseases, the amount of collagen deposited in the tissue is controlled by the balance between synthesis (regulated at the transcriptional and translational level) and degradation of different types of collagen components of the extracellular matrix (ECM) by matrix metalloproteinases (MMPs), which, at the same time, are regulated by tissue inhibitors of metalloproteinases [10]. In fibrosis, the positive balance for fueling collagen synthesis is influenced mostly by production of transforming growth factor β1 (TGF-β1), connective tissue growth factor (CTGF), interleukin-1, interleukin-6, platelet derived growth factor (PDGF), among other growth factors, which can be downregulated by PFD [11]. On the other hand, it has been clearly demonstrated by several groups, ours included, that PFD is able to upregulate MMPs-gene expression rendering an equilibrium of the tissue collagen amount.

Therefore, this study was aimed to evaluate the effects of PFD on wound healing, inflammation, and stricture formation and gene expression in the rat esophagus after caustic injury. Our results shown here suggest that PFD could be a suitable candidate for evaluation on esophageal healing in a given clinical scenario.

2. Material and methods

2.1. Materials

PFD was obtained from Cell Therapy and Technology (Mexico City, Mexico), in the form of an 8% crystalline gel. Ketamine was purchased from Virbac Inc (Carros, France). Primers and probes to perform real-time polymerase chain reaction were acquired from Applied Biosystems (Hammonton, NJ).

2.1.1. Animals

Male Wistar rats used in this study were obtained from Charles Rivers Inc (Boston, MA) weighing between 350 and 450 g were housed according to the principles and procedures outlined in the National Institute of Health's Guide for the Care and Use of Laboratory Animals. Rats were divided into three groups of six each.

2.2. Surgical procedure

After 12 h of fasting, rats were anesthetized with 50 mg/kg of ketamine hydrochloride given intraperitoneally. Two of the groups were subjected to caustic esophageal injury by following indications to reproduce the experimental model of Gehanno and Guedon [12]. Briefly, after a median laparotomy, 1-cm segment of the distal esophagus was exposed. A catheter was placed through puncturing a stomach anterior wall into the distal esophagus. Catheters were secured, and an isolated segment of distal esophagus was obtained. The distal catheter was clamped, and the isolated segment was distended by administering 1 mL of 32% NaOH under intraluminal pressure for 90 s until slight translucency of the esophageal wall and branching of the vessels was noted. Afterward, the distal catheter was unclamped and the esophagus rinsed with 10 mL of distilled water. The laparotomy was closed subsequently. All animals were kept on a standard rodent pellet diet with tap water *ad libitum* after surgery. One experimental group ($n = 6$) received treatment of 200 mg/kg of PFD 8% gel by gavage once daily for 21 consecutive days after caustic injury. A parallel group ($n = 6$) was treated in the exact surgical manner but rats were not administered with 8% PFD gel. The healthy group ($n = 6$) was not subjected to surgical procedure, not exposed to caustic injury, and did not receive PFD treatment. Contrast esophagograms and esophageal endoscopy were performed 21 d after caustic ingestion, and all animals were sacrificed with a high-dose of phenobarbital, esophageal tissue biopsy was obtained, and histology and molecular studies were performed.

2.3. Esophageal esophagogram

Esophageal contrast radiological study with barium was carried out after 21 d of treatment in all groups of rats. Percentage of stenosis was determined by measuring transversal diameter in both proximal area (nondamaged tissue) and stenosed and/or strictured distal (NaOH-damaged area) found in the same esophagus of each rat. Quantitative determination made by a computerized ImageJ program from National