

A preliminary exploration of pancreatic ferroptosis: Inhibition of ferroptosis alleviates pancreatic injury through the NLRP3 pathway in acute pancreatitis

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Short Report

Keywords: Acute pancreatitis, ferroptosis, lipid peroxidation, ROS, GPX4, NLRP3

Posted Date: May 12th, 2022

DOI: <https://doi.org/10.21203/rs.3.rs-1557525/v2>

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Additional Declarations: No competing interests reported.

Abstract

Objective The purpose of this study was to explore whether ferroptosis exists in acute pancreatitis (AP) and the effect of iron ions on the NLRP3 pathway.

Materials and Methods A total of 45 male C57BL/6 mice were randomly divided into three groups (control, AP, AP+2, 2'-bipyridyl). A total of 12 injections (caerulein, 50 µg/kg) were given at one-hour intervals. The mice in the AP+2, 2'-bipyridyl group were pretreated with 2, 2'-bipyridyl (20 mg/kg) for 1 hour and then injected with caerulein. The mice in the control group were injected with an equal volume of normal saline. All of the mice were killed one hour after the last injection. The pancreases were harvested for histopathological evaluation, immunohistochemistry analyses and western blotting. One-way ANOVA and Student's t test were performed. **Results** The ferroptosis inhibitor 2, 2'-bipyridyl can prevent the accumulation of iron ions, reduce the formation of lipid peroxides and alleviate injury in the process of AP, and reduce pancreatic inflammation through the NLRP3 pathway.

Conclusion This experiment revealed the presence of ferroptosis in AP. The application of 2, 2'-bipyridyl can obviously alleviate pancreatic damage through the NLRP3 pathway.

1. Introduction

In recent years, ferroptosis, a new mechanism of cell death, has aroused heated discussion [1]. Ferroptosis emphasizes the importance of iron ions to ROS. After Fe²⁺ aggregates, it can oxidize lipids and produce large amounts of ROS, causing cell death [2, 3]. There is also evidence that iron ions accumulate, GSH decreased and lipid peroxides accumulate in AP [3]. In addition, studies have shown that ROS produced in AP can activate NLRP3 [4]. Therefore, whether there is such a mode of death in AP and regulation of ferroptosis can have a therapeutic effect on AP should be further studied. In this study, an iron ion chelator was used to inhibit the aggregation of iron ions and block the pathway of ferroptosis to observe its influence on the occurrence and development of AP.

2. Materials And Methods

2.1 Animals and Reagents

A total of 45 male C57BL/6 mice were purchased and approved. Reagent information is detailed in the supplementary information.

2.2 Experimental Design in Mice

The mice were randomly divided into three groups (control, AP, AP + 2, 2'-bipyridyl). The specific operation method is presented in the summary section.

2.3 H&E Staining

H&E staining was used to examine the level of inflammation and tissue damage.

2.4 Measurement of Total Iron and GSH in the Pancreas

Iron and GSH were measured with a tissue iron and total glutathione assay kit.

2.5 Western Blot Analysis

The protocol for Western blotting has been described previously [5].

2.6 Immunohistochemical Staining

Paraffin-embedded tissue sections (5 μm) were processed through a series of procedures and stained with anti-NLRP3.

2.7 Statistical Analysis

The data are shown as the mean \pm SD of three independent experiments. One-way ANOVA and Student's t test were performed (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$).

3. Results And Discussion

When AP occurs, the oxidation and antioxidant systems are unbalanced, resulting in the production and accumulation of a large amount of ROS in the cell, which in turn lead to the production of lipid peroxides [3]. Ferroptosis underscores the importance of iron ions to ROS. In our study, compared to that in the control group, the iron content in the AP group increased, GSH decreased, and lipid peroxide increased significantly. After the application of divalent iron chelating agents, there was a significant improvement in these parameters (Fig. 1). Therefore, we believe that in the process of AP, an imbalance of oxidative stress caused by iron ion aggregation, the production of lipid peroxides, and ferroptosis are present. Through inhibition of the occurrence of ferroptosis, pancreatic damage during AP can be effectively alleviated (Fig. 5).

GPX4 plays a pivotal role in the occurrence of ferroptosis and is a key regulator. GPX4 converts GSH into oxidized glutathione and reduces cytotoxic lipid peroxides to the corresponding alcohols [3, 6]. Inside the cell, ferritin can serve as a reservoir of iron ions. When Fe^{2+} aggregates within cells, it is transported and stored in unstable iron pools and ferritin. Abnormal iron metabolic processes can trigger disturbances in intracellular function, prompting further ferroptosis [7]. Our study found that GPX4 and expression in the AP group were higher than those in the control group (Fig. 2A, 2C). Elevated GPX4 expression in AP improves the antioxidant capacity of tissue cells. However, due to the lack of its substrate GSH, the total antioxidant capacity remains unassailable. In addition, ferritin was higher in the AP group than in the control group, and the increase in ferritin content was positively correlated with the increase in iron ions (Fig. 2B). This finding is consistent with the fact that Fe^{2+} can be stored in ferritin when it aggregates within cells. The expression of GPX4 and ferritin following application of 2, 2'-bipyridyl decreased to a certain extent compared with that in the AP group.

The NLRP3 pathway has led to progress in the study of AP [8]. There is growing evidence that the inflammatory response is also activated when ferroptosis occurs. In this study, we found that the expression of NLRP3 decreased significantly in the AP + 2, 2'-bipyridyl group compared with the AP group (Fig. 3, 4). Its downstream pathway caspase-1/IL-1 β /TNF- α was also significantly different from that in the AP group. Our experiments confirmed that iron ion aggregation can regulate the occurrence and development of AP through the NLRP3 pathway. Inhibiting iron ions can effectively reduce the severity of AP.

Declarations

Ethics approval and consent to participate

Animals were approved by Institutional Animal Care and Use Committee of the First Clinical Medical College of Harbin Medical University (Harbin, China).

Availability of data and materials

All authors affirmed the authenticity of the experimental data.

Consent for publication

All the authors agreed to be published.

Conflict of interest

The authors declare no conflicts of interest.

Funding

This paper was supported by grants from the National Nature Scientific Foundation of China (No: 82070657), Applied Technology Research and Development Plan of Heilongjiang Province in China (NO: GA20C019), Outstanding youth funds of the first affiliated hospital of Harbin Medical University (HYD2020JQ0006) and Research projects of Chinese Research Hospital Association (Y2019FH-DTCC-SB1).

Authors' contributions

G.W and F.C designed the experiment. J.L and L.C did experiments and wrote articles. L.J, D.T, C.Y, X.G, and J.C offered help during the experiment.

Acknowledgments

This paper was supported by grants from the National Nature Scientific Foundation of China (No: 82070657), Applied Technology Research and Development Plan of Heilongjiang Province in China (NO: GA20C019), Outstanding youth funds of the first affiliated hospital of Harbin Medical University

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Figures

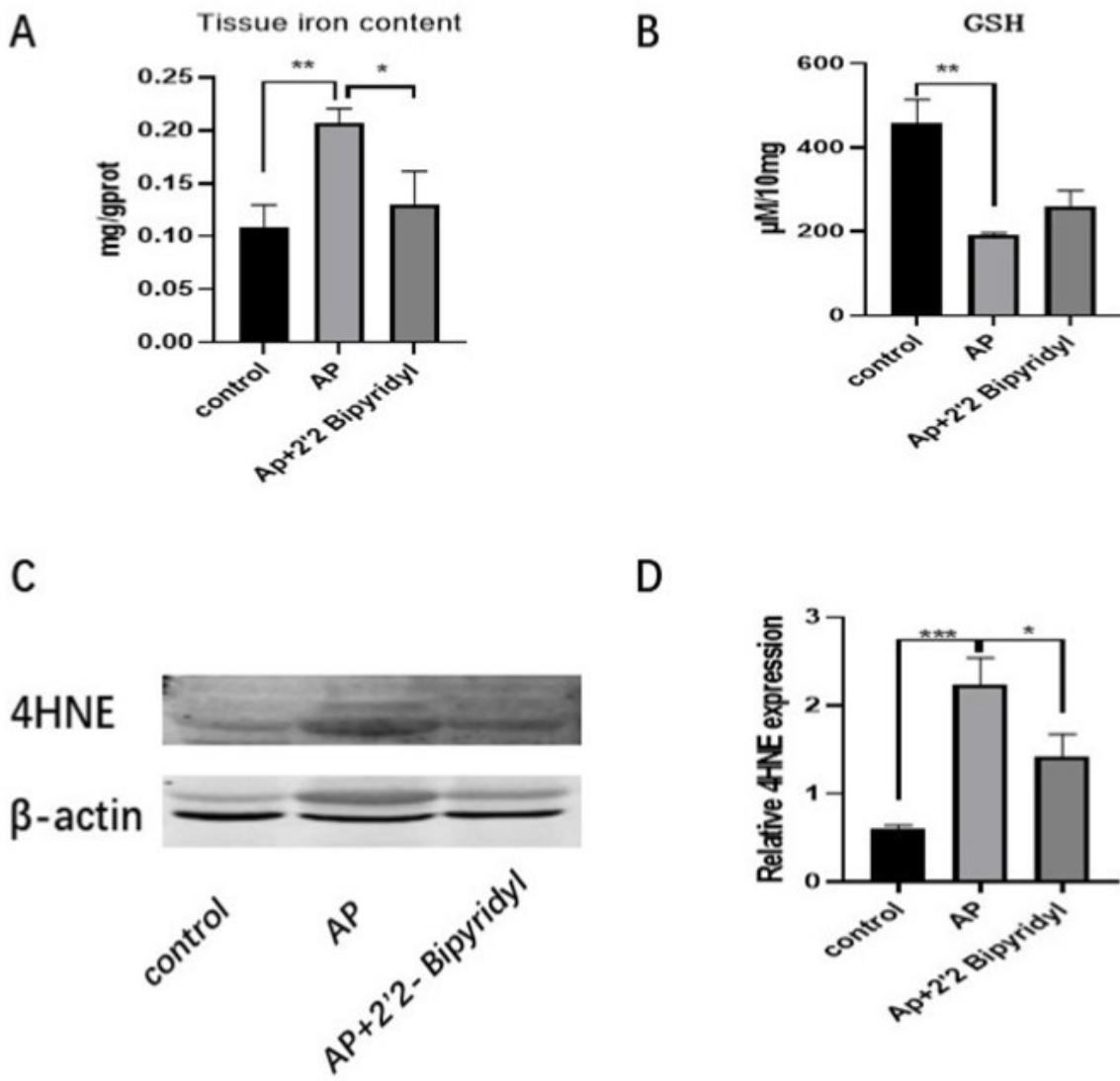


Figure 1

Figure 1

Expression of iron, GSH and 4HNE in mouse pancreas tissue in different groups Compared with the control group, iron increased significantly at AP($P<0.01$), GSH decreased significantly($P<0.01$), and 4HNE increased significantly($P<0.001$). Compared with AP group after medication, iron decreased($P<0.005$), GSH increased, and 4HNE decreased($P<0.005$).

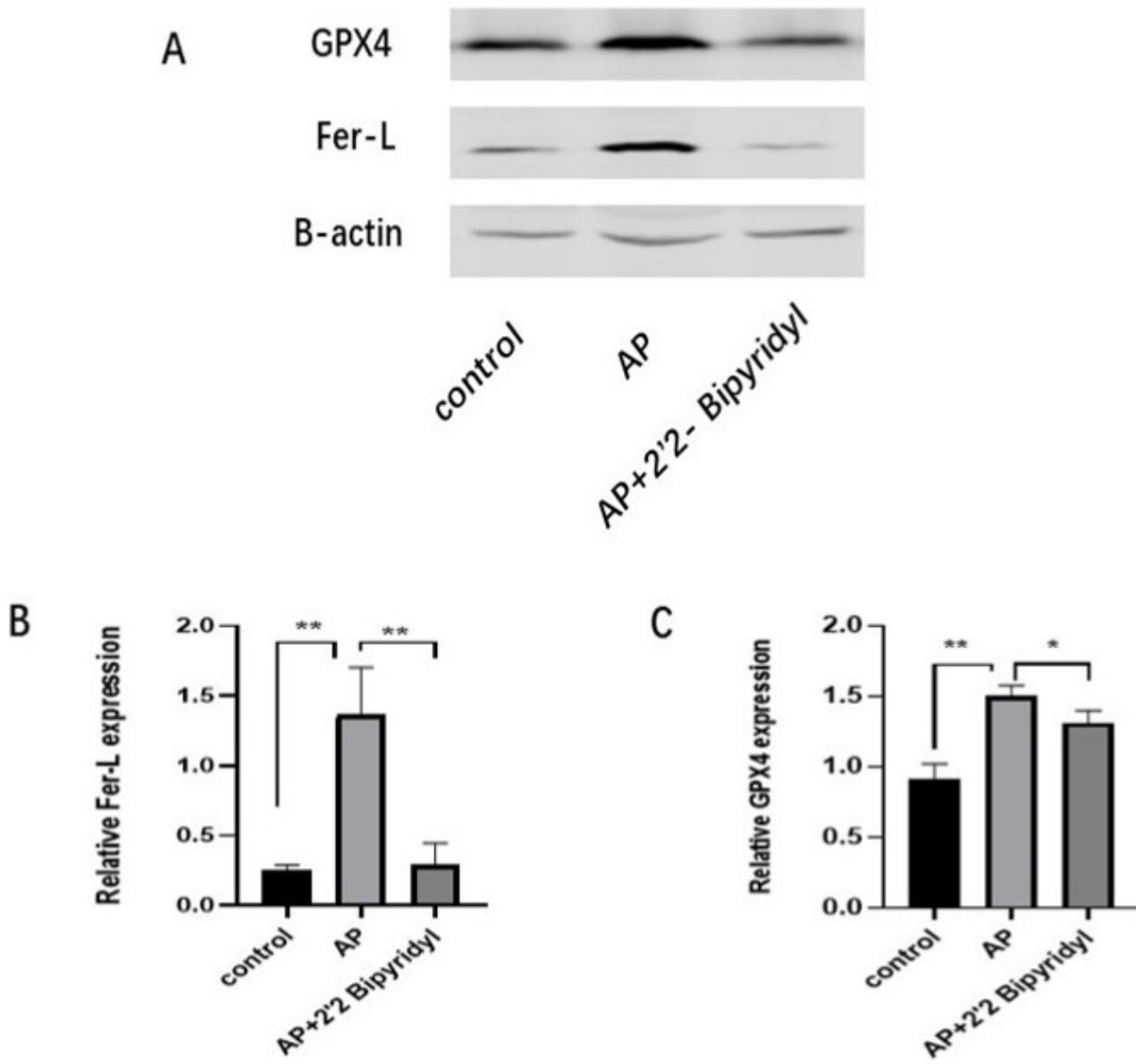


Figure 2

Figure 2

Expression of GPX4 and Fer-L in mouse pancreas tissue in different groups

Compared with the control group, GPX4 $P<0.001$, Fer-L $P<0.001$ in the AP group both increased. Compared with the AP group, GPX4 $P<0.001$, Fer-L $P<0.05$ in the AP+2'2'-Bipyridyl decreased to a certain extent.

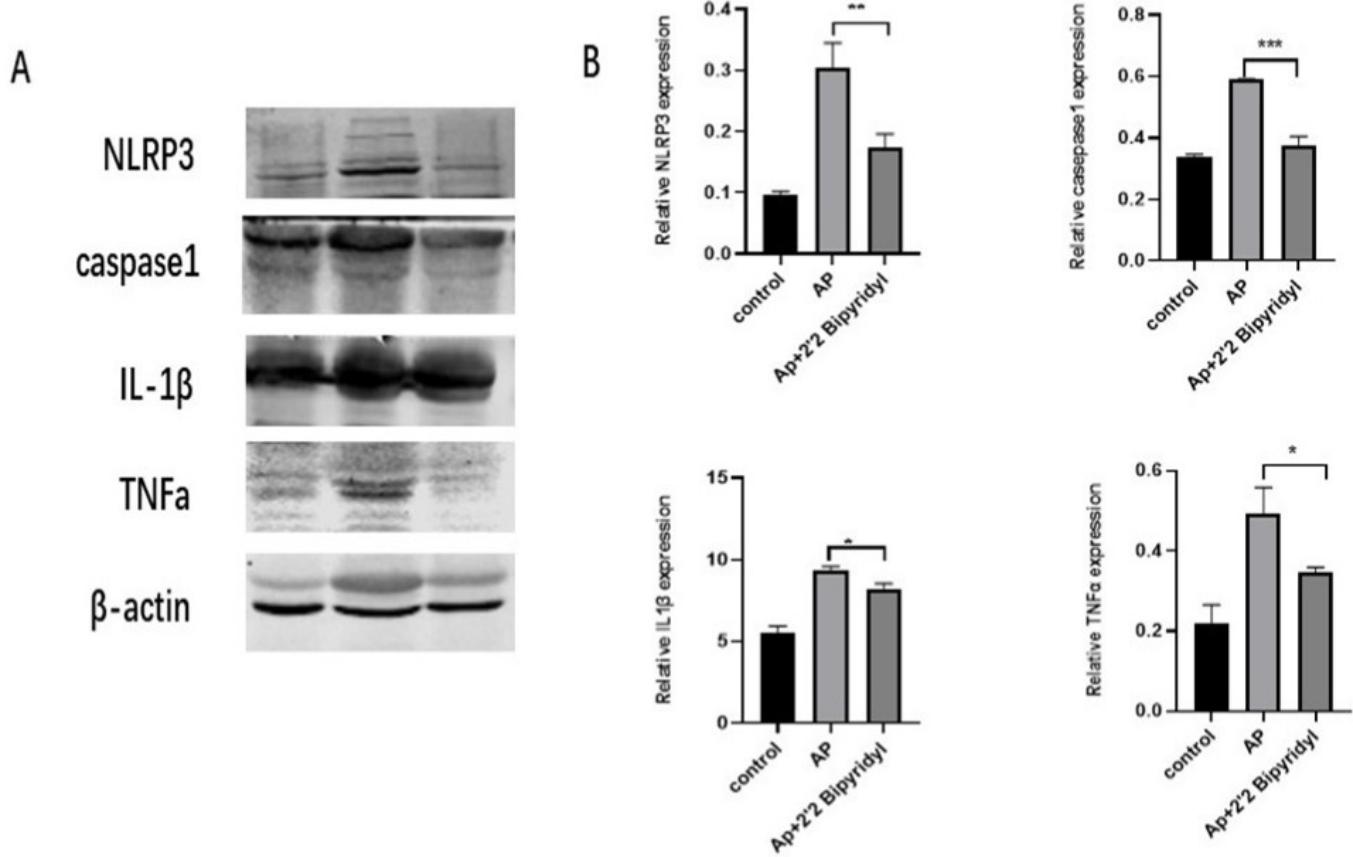


Figure 3

Figure 3

Expression of NLRP3, Caspase-1, IL-1 β and TNF α in mouse pancreas tissue in different groups

Compared with the AP group, NLRP3 P<0.01 , Caspase-1 P<0.001 , IL-1 β P<0.05 and TNF α P<0.05 in the AP+2'2- Bipyridyl group all decreased.

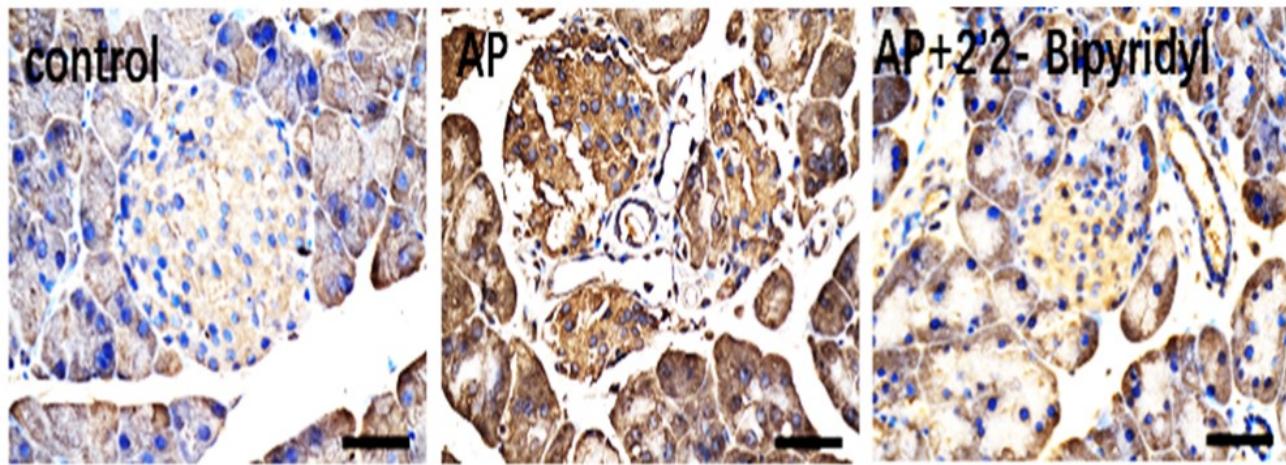


Figure 4

Figure 4

Immunohistochemistry of NLRP3

Compared with the AP group, the expression of NLRP3 in the 2 groups decreased.

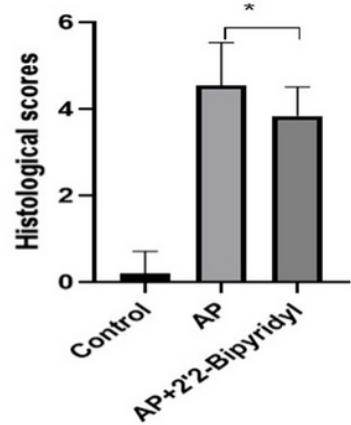
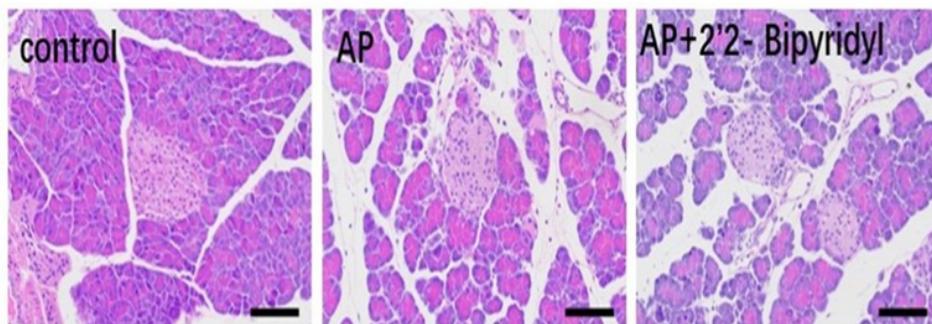


Figure 5

Figure 5

HE staining of pancreatic tissue

Compared with the AP group, the pancreatic tissue edema and inflammatory exudation of the 2 groups were reduced to a certain extent.

Supplementary Files

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