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Galactose protects pancreatic acinar cells from cerulein induced damage by regulating FGF21 and Klotho

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Abstract: To investigate the effect and mechanism of galactose on cerulean-induced pancreatic acinar cell injury. Acute pancreatitis cell injury model was established by arbusin-induced pancreatic acinar cell AR42J injury; galactose (25, 50, 100 mmol / L) was used to treat the injured cells, and the optimal concentration was 50 mmol / L; cell counting kit (CCK-8), enzyme linked immunosorbent assay (ELISA) to detect cell survival rate and necrosis rate; flow cytometry and Western blotting (Western blot) to detect cell apoptosis and autologous phage-related gene (Beclin1) and microtubule-associated protein 1 light chain 3 (LC3), apoptosis-related protein B-cell lymphoma / leukemia-2 (Bcl-2), Bcl-2-related X gene (Bax), and fibroblasts Expression of growth factor 21 antibody (FGF21) and anti-aging gene Klotho. A pancreatic acinar cell injury model was successfully established with cerana (100 nmol / L); galactose (25, 50, 100 mmol/L) In a concentration-dependent manner, the inhibitory effect of ceriferin on AR42J injury was inhibited at an optimal concentration of 50 mmol / L. Compared with the ceriferin group, the apoptosis rate of AR42J cells in the galactose group was significantly reduced. table Significantly increased, Bcl-2, FGF21 and Klotho protein expression was significantly increased, Bax protein was significantly decreased; the FGF21 inhibitor can be significantly reduced on galactose these caerulein-induced AR42J cells. Galactose can inhibit the apoptosis and autophagy of pancreatic acinar cells induced by cerana, and its potential mechanism is to up-regulate FGF21 and Klotho, providing a new potential drug for the treatment of acute pancreatitis.

Keywords: Galactose, Acute pancreatitis, FGF21, Klotho, Apoptosis, Autophagy

INTRODUCTION

Acute pancreatitis (AP) is an acute or chronic inflammation of the pancreas, which is characterized by premature activation of pancreatic acinar cells digestive enzymes, leading to pancreatic digest themselves. The prognosis of patients with acute pancreatitis depends to a large extent on the incidence of organ failure and necrosis of the infected pancreas, with an associated mortality of 15%-30%. Therefore, the treatment of pancreatitis drug research is of great importance. Fibroblast growth factors (FGF21) are hormones that control metabolism and cellular stress (Bruce 2020). FGF21 acts through FGF receptors to form tyrosine kinases with Klotho. Klotho is a one-way transmembrane protein anti-aging gene, which is co-expressed with FGF21 in the pancreas and is essential for digestive enzyme secretion of pancreatic acinar cells. It has recently been reported that FGF21 and Klotho play a role in the recovery process of pancreatitis. The aim of this study is to explore the relationship between the mechanism of D-galactose regulating apoptosis autophagy in pancreatic alveolar cells and FGF21 and Klotho (Das et al. 2019).

MATERIALS AND METHOD

Material

Rat pancreatic acinar cell AR42J was purchased from American type culture collection (ATCC); Hyroxacin (Item No.: C9026-1MG) was purchased from sigma; D-galactose (Item No.: 0637-100G) was purchased from Shanghai Lanji Technology Development Co., Ltd.; Lactate dehydrogenase (LDH); FGF21 inhibitor SU5402 was purchased from Gene Operation; Dimethylsulfoxide (DMSO) was purchased from Ybscience; The cell counting kit (CCK-8) was purchased from DOJINDO, Japan; Annexin V-fluorescein isothiocyanate/propidium iodide (Annexin V - FITC/PI) apoptosis detection kit was purchased from BestBio (Hollinger et al. 2019).

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Method

Cell culture

AR42J cells were cultured using RPMI 1640 medium (10% FBS) in a constant temperature incubator containing 5%CO2 at 37°C.

Establishment of acute pancreatic acinar cell injury model

AR42J cells were cultured for 12 h and treated with hygroxin (50, 100, 150 nmol/L) for 8 h, and then the optimal concentration of 100 nmol/L was screened out for the manufacturing of AP cell model.

Cell grouping and processing

Normal cultured AR42J cells without any treatment were labeled as the Blank group. The cerulein (50, 100 and 150 nmol/L) were diluted with PBS buffer, and then used for treating AR42J cells for 24 h,labeled as 50, 100 and 150 nmol/L groups, among which 150 nmol/L group was

labeled as cerulein group. D-galactose (25, 50, 100 mmol/L) was diluted with normal saline and then used to treat the cells in the cerulein group, labeled as 25 mmol/L galactose group, 50 mmol/L galactose group, and 100 mmol/L galactose group. The optimal concentration of 50 mmol/L galactose group was labeled as galactose group. Galactose group cells were treated with FGF21 inhibitor SU5402 (50 μmol/L) and DMSO (0.1%) for 3 h, and labeled as galactose+SU5402 group and galactose+DMSO group, respectively (Zhong et al. 2020).

CCK-8 test

Cells for cell detection were collected and adjusted to 105 /mL with medium. 100 μL of cell culture solution was added to the 96-well plate, and 20 μL of CCK-8 reagent was added, and placed at 37 to avoid light for 3 h. Cell absorbance (A490) was measured at wavelength of 490 nm. Cell survival rate is A490 sample/A490control×100%.

ELISA test

The cells were treated and tested according to the instructions of the lactate dehydrogenase (LDH) assay kit.

Flow cytometry

The cells to be tested were collected, washed with PBS, and suspended with a binding buffer. According to the instructions for Annexin V-FITC/PI kit, Annexin V-FITC and PI were added for reaction in a light-avoiding manner for several minutes, and then flow cytometry analysis was performed immediately after the reaction. The total apoptosis rate was equal to the sum of Annexin V-FITC staining positive rate and PI staining positive rate (Kim et al. 2019).

Western blot test

Cells in the logarithmic phase were collected and fully lysed with sufficient lysates to extract the total protein. After quantitative denaturation, the supernatant was collected for SDS-PAGE protein electrophoresis. The albumen were wetted onto the PVDF membrane with a membrane rotator, and then sealed with 5% skim milk for 2 h. The membrane was incubated overnight at 4 with a diluted primary antibody solution (1:5000-1500), and then transferred to a doubled diluted secondary antibody solution (1:1000) for 1 hour at 37 . The membrane was exposed with a super sensitive ECL luminescence kit (Wu et al. 2019).

STATISTICAL ANALYSIS

All data in the experiment were analyzed by the special statistical software PEMS3.2, and the measurement data were expressed as mean ± standard deviation. One-way ANOVA and intra-group pairwise comparison SNK-q test were used for data comparison between multiple groups, and independent sample t test was used for comparison between two groups. The difference was considered statistical significant when $p<0.05$ (Liu & Cui 2019).

RESULTS

Establishment of cerulein-induced acute pancreatic acinar cell injury model

As shown in table 1, compared with the Blank group, the survival rate of AR42J cells treated with cerulein (50, 100, 150 nmol/L) was significantly decreased in concentration dependence, while the necrosis rate was significantly increased in concentration dependence ($p<0.05$). The 100 nmol/L of cerulein was used for the manufacturing of AP acinar cell damage model and for subsequent experimental studies (Ma & Ren 2019).

Table 1: Survival rate and necrosis rate of pancreatic acinar cells treated with cerulein at different concentrations (x±s, n=9)

Group	Survival rate (%)	Necrosis rate (%)
Blank	99.34±6.12	3.14±0.12
50 nmol/L	92.65±8.14	4.61±0.31
100 nmol/L	60.82±3.42*	31.50±2.11*
150 nmol/L	32.12±2.01*	50.98±4.34*
F	290.023	813.888
p	0.000	0.000

*p<0.05, compared with Blank group.

Table 2: Regulation of galactose on proliferation of AP pancreatic acinar cells (x±s, n=9)

Group	Survival rate (%)	Necrosis rate (%)
cerulein	62.85±3.47	30.78±2.14
25 mmol/L galactose	64.59±4.41*	28.48±2.21*
50 mmol/L galactose	82.98±6.84*	12.59±1.12*
100 mmol/L galactose	85.74±6.91*	8.87±0.69*
F	41.072	393.176
p	0.000	0.000

*p<0.05, compared with cerulein group.

Regulation role of D-galactose on proliferation of AP pancreatic acinar cells

As shown in table 2, compared with the cerulein group, the survival rate of AP pancreatic acinar cells treated with D-galactose (25, 50, 100 mmol/L) increased in a concentration dependence, while the necrosis rate decreased in a concentration dependence ($p<0.05$). D-galactose with an intermediate concentration of 50 mmol/L was selected for subsequent experimental study.

Regulation role of D-galactose on apoptosis of AP pancreatic acinar cells

As shown in fig. 1 and table 3, compared with the cerulein group, the apoptosis rate of AP pancreatic acinus cells in the galactose group was significantly decreased, the expression of Bcl-2 protein was significantly increased,

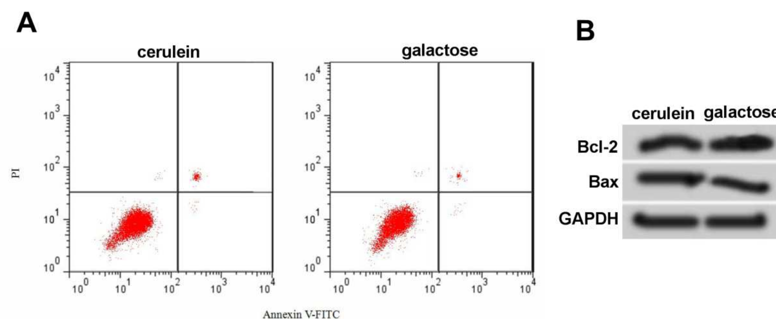

Fig. 1: Apoptosis of AP pancreatic acini cells treated with galactose and electrophoresis of Bcl-2 and Bax protein expression

Table 3: Regulation of D-galactose on apoptosis of AP pancreatic acinar cells (x±s, n=9)

Group	Apoptotic rate (%)	Bcl-2 protein	Bax protein
Cerulein	26.41±1.98	1.00±0.06	0.99±0.07
Galactose	13.42±1.10*	1.35±0.09*	0.69±0.05*
t	17.205	9.707	10.462
p	0.000	0.000	0.000

*p<0.05, compared with cerulein group

and the expression of Bax protein was significantly decreased ($p<0.05$). Therefore, D-galactose can inhibit cerulean-induced apoptosis of pancreatic acinar cells (Wang & Zhang 2019).

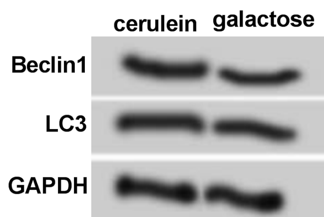

Fig. 2: Electrophoresis of autophagy-related proteins in AP pancreatic acinar cells treated with galactose

Table 4: Expression of autophagy-related proteins in galactose-treated AP pancreatic acinar cells (x±s, n=9)

Group	Beclin1 protein	LC3 protein
Cerulein	1.02±0.07	1.00±0.08
Galactose	0.59±0.04*	0.65±0.05*
t	16.000	11.130
p	0.000	0.000

*p<0.05, compared with cerulein group.

Regulation of autophagy in AP pancreatic acinar cells by d-galactose

As shown in fig. 2 and table 4, compared with the cerulein group, the expression levels of autophagy related proteins Beclin1 and LC3 in AP pancreatic acinus cells in galactose group were significantly decreased ($P<0.05$). It can be seen that D-galactose can inhibit cerulein induced autophagy of pancreatic acinar cells.

Regulation of the expressions of FGF21 and Klotho in AP pancreatic acinar cells by D-galactose

As shown in fig. 3 and table 5, compared with the cerulein group, the protein expression levels of FGF21 and Klotho in AP pancreatic acinus cells of the galactose group were significantly increased ($p<0.05$). It can be seen that D-galactose can up-regulate the expressions of FGF21 and Klotho in the pancreatic acinar cells induced by cerulein.

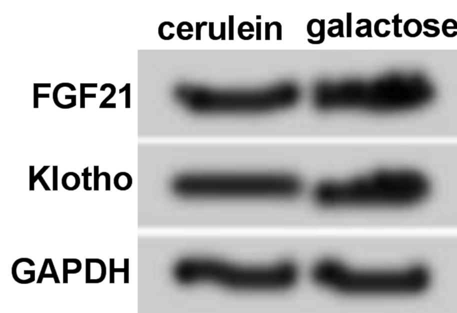

Fig. 3: Electrophoresis of FGF21 and Klotho protein expression in galactose-treated AP pancreatic acinar cells

Table 5: Regulation of the expressions of FGF21 and Klotho in AP pancreatic acinar cells by D-galactose (x±s, n=9)

Group	FGF21 protein	Klotho protein
Cerulein	1.00±0.08	1.01±0.06
Galactose	1.42±0.10*	1.35±0.11*
t	9.839	8.140
P	0.000	0.000

*P<0.05, compared with cerulein group

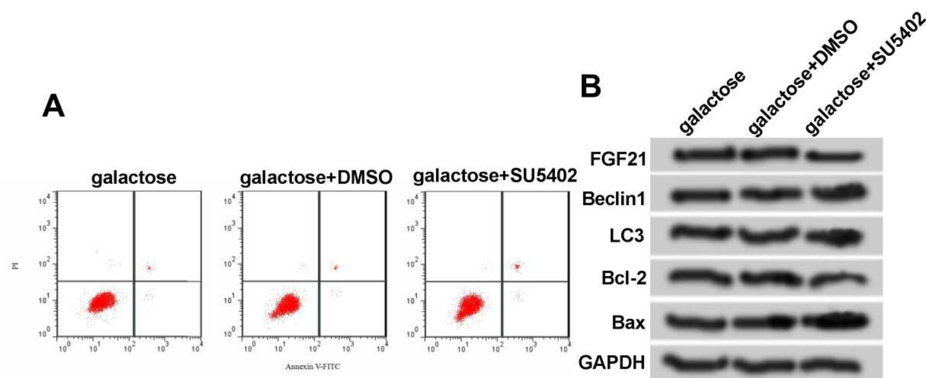

Fig. 4: Electrophoretogram of protein expression of FGF21 inhibitor and apoptosis on the effect of galactose on AP pancreatic acinar cells

Table 6: Effect of FGF21 inhibitor on the regulation of AP-pancreatic acinar cell survival, apoptosis and autophagy by D-galactose (x±s, n=9)

Group	FGF21 protein	Survival rate (%)	Apoptotic rate (%)	Beclin1 protein	LC3 protein	Bcl-2 protein	Bax protein
Galactose	0.98±0.07	82.69±4.19	12.98±1.10	0.99±0.06	0.98±0.06	1.01±0.07	1.02±0.07
Galactose +DMSO	0.99±0.06	83.02±6.31	13.12±1.11	1.00±0.07	1.00±0.05	0.98±0.05	0.99±0.07
Galactose +SU5402	0.31±0.02*	53.45±4.25*	18.97±1.26*	1.42±0.13*	1.61±0.14*	0.66±0.05*	1.45±0.12*
F	21.711	103.17	78.306	1.019	176.100	102.636	73.897
p	0.000	0.000	0.000	0.000	0.000	0.000	0.000

*p<0.05, compared with galactose+DMSO group

Effect of FGF21 inhibitor on the regulation of AP-pancreatic acinar cell survival, apoptosis and autophagy by D-galactose

As shown in fig. 4 and table 6, compared with the galactose+DMSO group, the expression of FGF21 protein in the galactose+SU5402 group was significantly decreased, the cell survival rate was significantly decreased, and the cell apoptosis rate was significantly increased, the expressions of FGF21 and Klotho were significantly increased, the expression of Bcl-2 protein was significantly decreased, and the expression of Bax protein was significantly increased (P<0.05). Therefore, FGF21 inhibitor can partially weaken the regulation role of D-galactose on cerulein induced survival, apoptosis and autophagy of pancreatic acinar cells.

DISCUSSION

D-galactose can promote aerobic metabolism and participate in mitochondrial dysfunction in human primary myoblast cells. Studies have shown that substituting D-galactose solution for drinking water can significantly reduce all pathological scores of patients with asparaginase and alcohol-metabolization-induced AP, making D-galactose a potentially important supplement for AP treatment. Recently, Xie et al. reported that D-galactose significantly reduced the loss of adenosine triphosphate (ATP) and pancreatic necrosis in AP mouse models, and D-galactose significantly reduced the necrosis and inflammation of pancreatic acinar cells in vitro (Xie et al. 2020). In this study, D-galactose was found to improve the survival rate of AR42J cells and reduce the necrosis rate of AR42J cells induced by cerulein, thus showing a certain effect on protecting cells from being damaged. Further studies showed that D-galactose can inhibit apoptosis and autophagy in damaged AR42J cells, which suggests that D-galactose can inhibit cerulein induced apoptosis and autophagy of AR42J cells, and thus playing a protective role. The results were initially validated in vitro and not in vivo or clinically.

FGF21 interacts with a single transmembrane protein β-Klotho by acting on a cell surface receptor composed of FGFR with tyrosine kinase-active components. FGF21, β-klotho and FGFRs were co-expressed in mouse pancreas. Recently, Li et al. found that the foreign body of mesenchymal stem cells overexpressing Klotho reversed cerulein induced apoptosis of AR42J cells and activated NF-kB in cells, and Klotho on the other hand reduced the severity of pancreatic inflammation after penicillin treatment (Li et al. 2020). This suggests that Klotho is a potential therapeutic target for clinical interventions for acute pancreatitis. Huang et al. reported that mice lacking FGF21 would accumulate zymogen granules and were

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susceptible to the effects of pancreatic endoplasmic reticulum stress, and mice with acinar cell-specific βklotho deficiency would also accumulate zymogen granules (Huang et al. 2020). This suggests that FGF21 is a digestive enzyme secretin whose physiological function is to maintain the protein homeostasis of acinar cells. This study found that the expressions of FGF21 and Klotho were abnormally increased in damaged AR42J cells after D-galactose treatment, suggesting the potential of D-galactose in the treatment of pancreatic injury, and again demonstrating the protective role of FGF21 and Klotho in pancreatic injury. This result is consistent with previous studies on the function of FGF21 and Klotho in pancreatitis. Further exploration has found that FGF21 inhibitor can weaken the protective effect of D-galactose on injured pancreatic cells, which further indicates that FGF21 and Klotho play an important role in the mechanism of D-galactose's therapeutic function.

CONCLUSION

In conclusion, D-galactose can inhibit cerulein induced apoptosis and autophagy of pancreatic acinar cells and play a protective role, providing a theoretical reference for the treatment of acute pancreatitis by D-galactose.

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