ORIGINAL ARTICLE

Comparable Responses in Male and Female Mice to Cerulein-Induced Chronic Pancreatic Injury and Recovery

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ABSTRACT

Objective The cerulein-induced mouse pancreatitis model is a well-established, commonly used representation of human chronic pancreatitis pathology. Although studies report sex-dependent differences in human chronic pancreatitis, there are no studies in this model directly comparing sex response to pancreatic injury and recovery. Therefore, we designed a study to investigate whether sex-dependent differences in chronic pancreatitis injury and recovery exist in the cerulein-induced pancreatitis model. **Methods** Adult male and female C57BL/6 mice were administered cerulein (50 µg/kg, 5 hourly intraperitoneal injections/day, 3 days/week) for 4 weeks to induce chronic pancreatitis; control mice received normal saline injections. Pancreata and blood were harvested at 4 days (as injury group) or 4 weeks (as recovery group) after the last injection. Amylase secretion was measured from the serum. Acinar injury was scored on H&E sections. Fibrosis was assessed by Sirius Red and collagen immunofluorescence staining. **Results** Compared to time-matched controls, injury group displayed decreased body and pancreas weight, and increased acinar injury and fibrosis, with no significant differences between males and females. Recovery group demonstrated recovery of body weight, partial recovery of pancreas weight, reversal of acinar injury, and partial reversal of fibrosis, with no significant differences between males and females. Amylase secretion/body weight was similar across all groups. **Conclusions** Male and female mice of the cerulein-induced chronic pancreatitis demonstrate similar responses to chronic pancreatitis injury and recovery. Although this model may not sufficiently emulate sex-dependent responses in human chronic pancreatitis, our study supports that both sexes of mice from this model can be used for the study of chronic pancreatitis.

INTRODUCTION

Chronic pancreatitis (CP) is a progressive inflammatory and fibrotic disease, resulting in long term exocrine and endocrine insufficiency due to extensive pancreatic injury [1]. Clinically, CP patients present with chronic abdominal pain, weight loss and diarrhea, and are at an increased risk of morbidity from complications such as malnutrition, diabetes, and pancreatic cancer [2, 3]. Pain management and supportive care are the only current therapies existing for CP patients. Despite decades of research, no specific treatment modality is available for halting the progression of the disease, largely due to an incomplete understanding of its underlying mechanisms [4, 5].

Although ideal, access to human pancreatic tissue is limited during pancreatitis development, thus animal models serve an essential role in the study of pancreatitis pathogenesis and treatment [6]. The cerulein-induced rodent model is the most widely used for acute and chronic pancreatitis studies [7]. Recapitulating similar pathology to human pancreatitis, this model is non-invasive, time-dependent, and highly reproducible [8]. However, like any animal model, limitations exist in paralleling cerulein-

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induced pancreatitis to human disease. For instance, excessive secretagogue stimulation may not be a clinically relevant cause of pancreatitis and studies have shown that, unlike the mouse, whether or not cholecystokinin receptors are present in the human pancreas is still uncertain [7, 8, 9, 10, 11, 12]. Even with such limitations, there is no overt pathological difference between human and cerulein-induced mouse CP.

Discrepancies of sex susceptibility exist in many diseases: more frequent and severe irritable bowel syndrome occurs in female patients, while the opposite is true for colorectal cancer, with males at a higher risk [13, 14]. Human studies have reported a higher incidence and prevalence of CP in male patients [4, 15, 16, 17, 18, 19], which is likely due to the higher prevalence of alcohol consumption in males, as heavy drinking has been linked to the development of pancreatitis [4, 17, 20, 21]. However, less than 5% of patients with significant alcohol consumption develop CP [22, 23], suggesting a multifactorial etiology exists in pancreatitis development.

In animal models of pancreatitis, inconsistent differences are reported in disease susceptibility and severity between males and females. For example, in mice with genetically impaired autophagy, CP development was more pronounced in males than in females [24, 25]. While in a choline-deficient diet enriched with ethionine model, female mice experienced more severe forms of AP than males [26, 27, 28]. However, there are no studies directly comparing male and female response to pancreatic injury and recovery in the cerulein-induced mouse model. The rationale of this study was to investigate whether sex-dependent differences in chronic pancreatitis injury and recovery exist in the cerulein-induced pancreatitis mouse model. The outcome from this study may influence future animal study design and data interpretations for the cerulein-induced pancreatitis model.

To examine potential sex-dependent responses to CP in the cerulein-induced mouse model, we designed a study in which both male and female adult C57BL/6 mice were injected with cerulein for a period of 4 weeks, followed by 4 weeks of withdrawal from cerulein, to mimic CP injury and recovery. Following CP injury, we found that both sexes recovered from loss of body weight and acinar injury, and partially recovered from pancreas weight loss and fibrosis. Overall, we observed similar responses in CP injury and recovery in both male and female mice.

METHODS

Reagents

Cerulein, a cholecystokinin analog and secretagogue, was purchased from Bachem Americas (Torrance, USA). Phadebas Amylase test tablets were purchased from Fisher Scientific (Pittsburgh, USA). Direct Red 80 and picric acid for Sirius Red staining were purchased from Sigma Aldrich Corporate (St. Louis, USA). Antibody against collagen type I (Col1) was purchased from Abcam (Cambridge, USA).

CP Model

Adult C57BL/6 mice, purchased from the Jackson Laboratory (Bar Harbor, USA), were housed in a climate-controlled room with ambient temperature of 23°C and a 12:12-h light-dark cycle. Animals were fed standard laboratory chow and given water ad libitum. For the study, animals were randomly assigned to control or experimental CP groups and randomly assigned to either recovery or injury group. A total of 40 mice were included in the study, 20 in control and 20 in experimental group, with an equal gender ratio of male:female. For CP induction, the mice received cerulein injections (50 μg/kg, intraperitoneal (ip), 5 hourly injections/day, 3 days/week) for 4 weeks. The control mice received normal saline injections [29, 30, 31]. Animal body weights were recorded weekly. Pancreata were harvested 4 days (designated as injury group) or 4 weeks (designated as recovery group) after the last injection and weighed. Previous animal studies have used recovery periods of 3 or 5 weeks after injury and demonstrated a partial recovery of CP [32, 33]. Based on the literature, in this study, a recovery period of 4 weeks was designated from the completion of the last cerulein injection to tissue harvesting for analysis. At the designated time points, the mice were euthanized, and the blood and pancreas were harvested. All procedures are depicted in **Figure 1**. The mouse serum was collected for amylase testing and the pancreatic tissue samples were fixed in 10% formalin and embedded in paraffin for morphological studies.

Amylase Measurement

Serum amylase levels were determined using the Phadebeas Amylase test tablets as instructed by the manufacturer.

Morphological Examination

The pancreatic tissue sections (5 μm-thick) were prepared and hematoxylin-eosin (H&E) staining was performed. A semi-quantitative histological scoring system on severity of chronic acinar lesions was conducted by an experienced pathologist blinded to the sample identity. Morphological changes were scored on the extent of acinar injury including abnormal architecture, glandular atrophy, and pseudo tubular complexes [30, 34].

Analysis of Pancreatic Fibrosis

Sirius Red staining was performed on pancreatic sections for collagen deposition [29, 31, 32]. Immunofluorescence (IF) staining was performed for Collagen 1 (Col1) expression using antibody against Col1. Seven non-overlapping images were captured from each section/pancreas and quantified with NIS-Elements Br 3.0 software. The stained areas were calculated as a percentage of total tissue area analyzed [29, 31].

CP Diagnosis Criteria

The criteria for diagnosis of CP in mice include the semi-quantitative histological scoring system on severity of

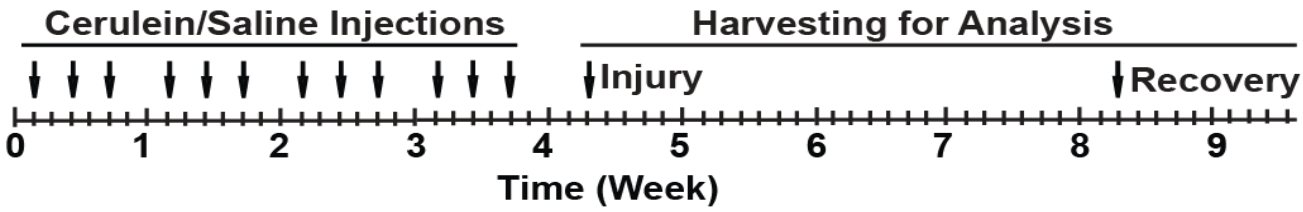

Figure 1. A schematic presentation of protocol. Arrows indicate the time points of injections or tissue harvesting.

chronic acinar lesions, and the quantitative evaluation on fibrosis by measuring collagen deposition and production [30,34]. Compared to time matched control, mice with significant increases of histological scores and fibrosis in the pancreas were diagnosed as having CP.

Ethics

Animal experiments were performed according to the protocol number AWC-14-0072 approved on May 29, 2014 by the Animal Welfare Committee of the University of Texas Health Science Center at Houston.

Statistics

Data are expressed as mean ± SEM. Differences between two groups were analyzed using Student's t-test, differences among multiple groups were analyzed using One Way ANOVA, and differences of body weight were analyzed using One Way Repeated Measures ANOVA (SigmaPlot, Systat Software, San Jose, USA). P value <0.05 is considered significant.

RESULTS

Recovery of Body Weight, Pancreas Weight, Amylase Secretion, and Acinar Morphology Following CP Injury

As an overall functional assessment, animal body weights were recorded weekly during CP injury and recovery stages. Male mice in the CP group gained less body weight than their time matched controls during weeks 3-4 of the injury stage and gradually gained similar body weight compared to their time matched controls during the recovery stage. Female mice in the CP group gained less weight than their time matched controls during weeks 2-4 of the injury stage and up to week 2 of the recovery stage, then gradually gained similar body weight compared to their time matched controls by the end of the recovery stage **(Figure 2)**.

Pancreas weights were also recorded at harvest and reported as pancreas/body weight ratio. As shown in **Figure 3**, the ratio of pancreas/body weight of both male and female mice in CP injury groups reduced 60% compared to time matched controls (male p=0.002, female p=0.011) and recovered approximately 12% in CP recovery groups (male p=0.024, female p=0.032). No differences were noted between males and females **(Figure 3)**.

To evaluate the pancreatic exocrine function, we measured amylase secretion from the mouse serum. We found that in all groups, male mice had higher amylase secretion than that of female mice **(Figure 4a)**. However, after normalized by respective body weight, the differences were only observed in CP4w group **(Figure 4b)**. These results suggest that body weight should be taken into consideration when interpreting amylase secretion data of male and female mice. These data also confirm that amylase secretion does not correlate with chronic pancreatic injury.

Pancreatic acinar injury was evaluated based on histopathological changes. Compared to controls, both male and female CP injury groups showed typical histological changes in the pancreas with increased histopathological scores (male and female p=0.001). No significant difference in acinar injury was observed between males and females. The acinar injury recovered completely after 4 weeks in all mice except one male mouse with the minimal histoscores. However, no significant differences were observed in this group between male and female mice **(Figure 5)**.

Partial Recovery of Pancreas Fibrosis Following CP Injury

Extensive pancreatic fibrosis is a hallmark of CP. To examine the extent of pancreatic fibrosis in cerulein-induced CP injury mice, we used Sirius Red staining to assess collagen deposition and immunofluorescence staining for Col1 protein expression. After 4-week CP induction, respective to male and female mice, there was a 16- and 11-fold increase in collagen deposition compared to time matched controls (male p=0.005, female p=0.004). After 4-week recovery following CP injury, males and females showed 50% and 27% reversal of collagen deposition (male p=0.014, female p=0.009), respectively. No significant differences were observed between males and females **(Figure 6)**.

For the Col1 IF assay, compared to their time matched control groups, males and females showed 23- and 30-fold increase of Col1 expression, respectively (male p=0.013, female p=0.033). After 4-week recovery following CP injury, males and females showed 70% and 63% reversal of Col1 expression, respectively (male p=0.016, female p=0.024). No significant differences were observed between males and females **(Figure 7)**.

Taken together, our data demonstrate that pancreatic acinar injury reversed at 4 weeks after cessation of cerulein injections, while pancreatic fibrosis and pancreas/body weight only recovered partially at the same time point. Throughout the entire experiment, there were no significant differences between males and females.

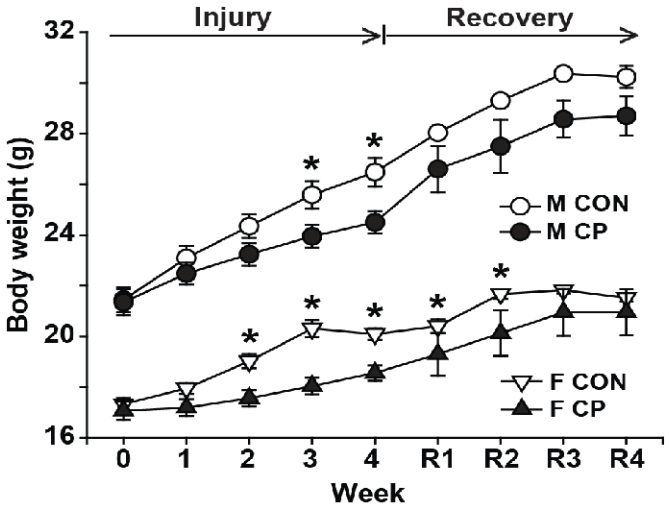

Figure 2. Mouse body weight over the CP injury and recovery stages. C57BL/6 mice, both male (M) and female (F), received cerulein injections (50 μg/kg, ip, 5 hourly/day, 3 days/week) for 4 weeks for CP injury period (0-4), followed by cessation of cerulein injections for 4 weeks for recovery period (R1-R4). Time-matched control mice received normal saline injections (CON). Mouse body weight was recorded weekly, and presented as mean±SEM from each group. n=10 mice/sex in injury groups, n=4 mice/sex in recovery groups. *p<0.05 CP *vs.* the time-matched controls for each time point respective to males and females.

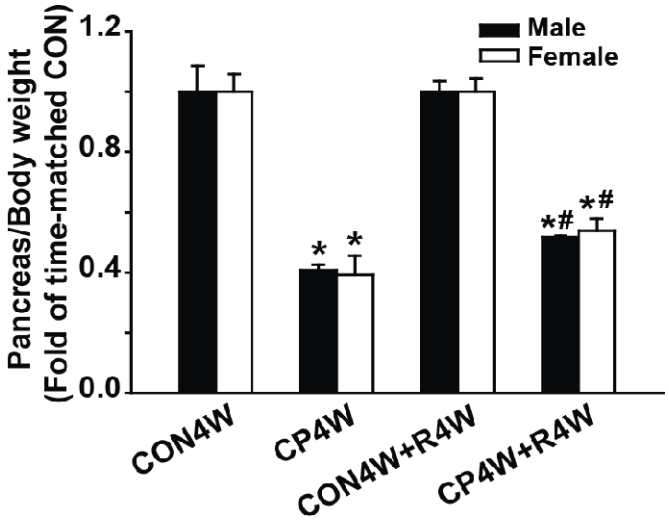

Figure 3. Ratio of pancreas/body weight at CP injury and recovery. Injury group pancreata were harvested 4 days after cerulein injections (CP4W) or normal saline injections (CON4W) and recovery group pancreata were harvested 4 weeks after cessation of cerulein injections (CP4W+R4W) or normal saline injections (CON4W+R4W) as described in Figure 2. Pancreas weight was recorded and normalized with corresponding body weight. The ratio of pancreas/body weight is presented as mean±SEM from each group/sex. n=6 mice/sex in injury groups, n=4 mice/sex in recovery groups. *p<0.05 *vs.* time-matched controls. #p<0.05 compared the differences between CP4W and CP4W+R4W after normalized against their respective time-matched controls.

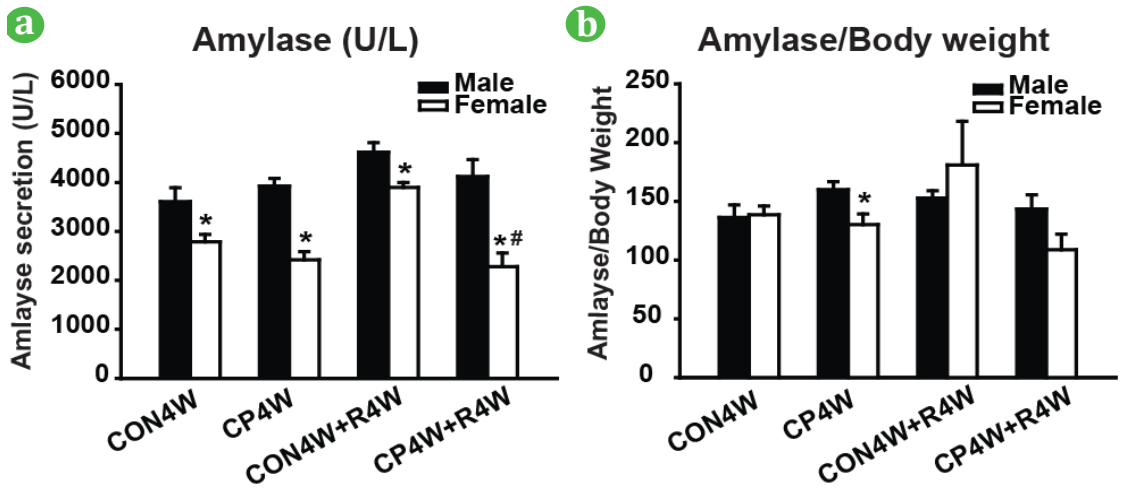

Figure 4. Amylase secretion levels at CP injury and recovery. The mouse serum was collected from the above mentioned mice. Amylase levels were measured and presented as mean±SEM from each group/sex. n=6 mice/sex in injury groups, n=4 mice/sex in recovery groups. **(a).** Serum amylase levels (U/L). **(b).** Ratio of amylase/body weight. *p<0.05 *vs.* male mice, #p<0.05 *vs.* time matched control.

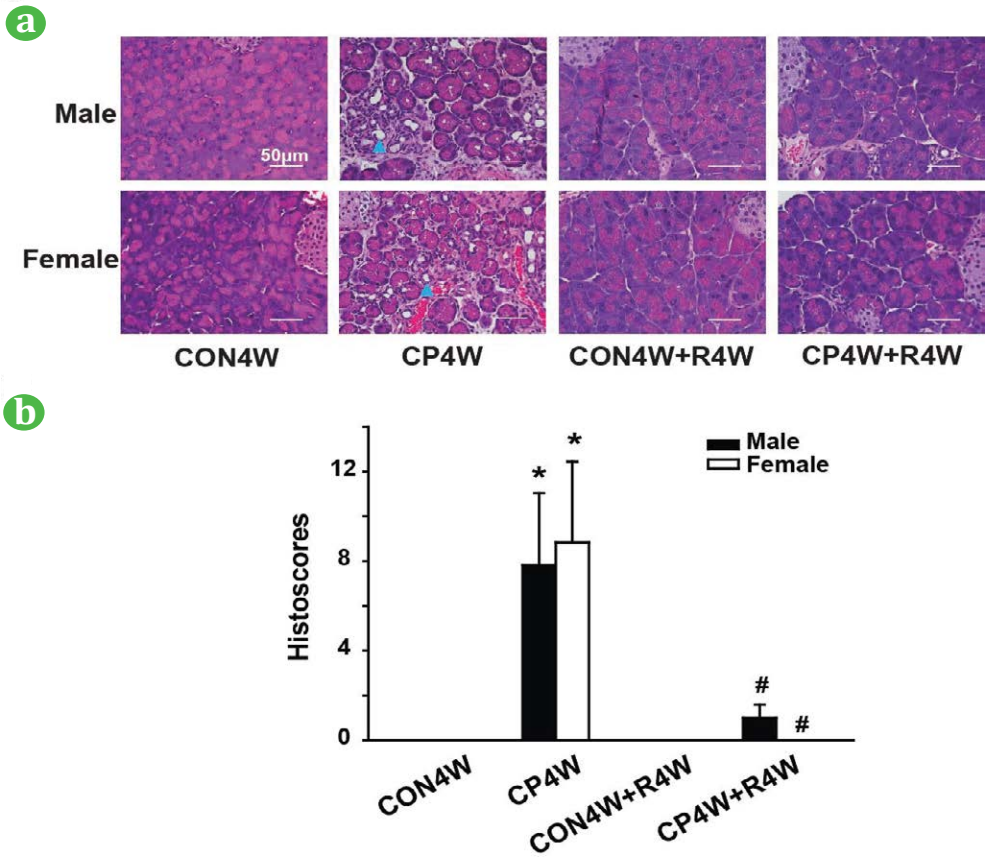

Figure 5. Pancreatic acinar injury at CP injury and recovery stages. Pancreatic tissue sections were prepared from the above mentioned pancreata. H&E staining was performed. **(a).** Representative images of H&E staining. Blue arrow heads point to pseudo-tubular complexes. **(b).** Total histopathological scores are presented as mean±SEM from each group/sex. n=6 mice/sex in injury groups, n=4 mice/sex in recovery groups. *p<0.05 *vs.* time-matched controls. #p<0.05 *vs.* CP4W.

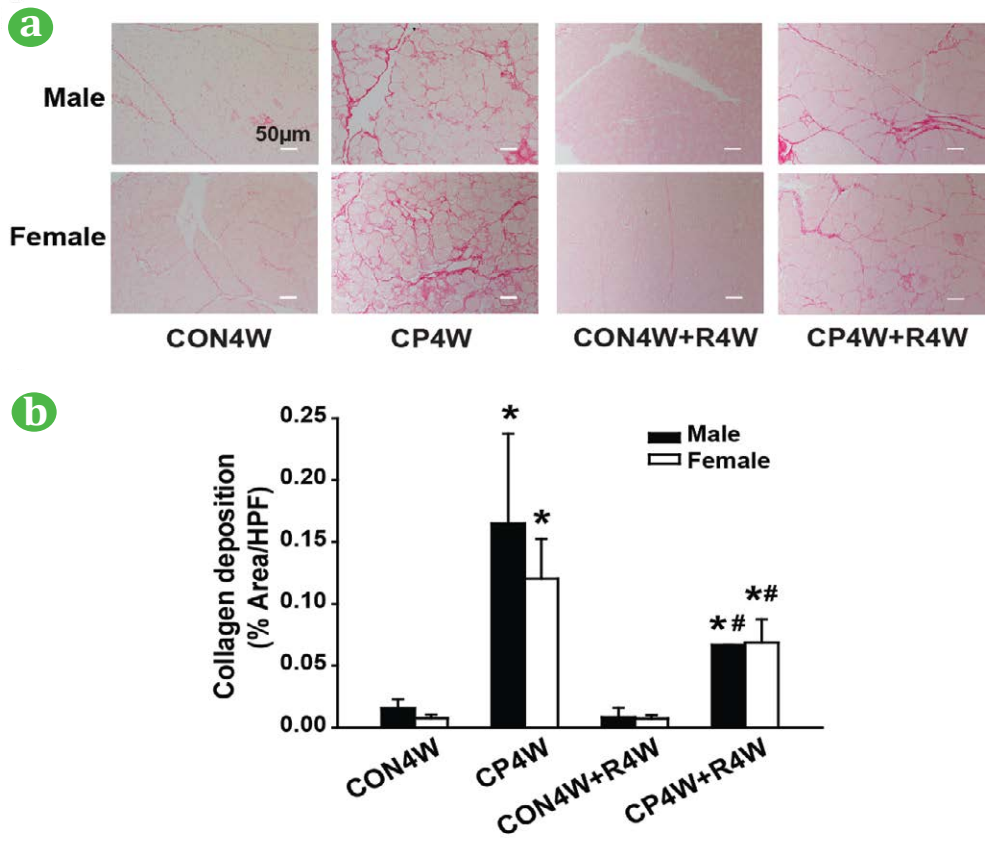

Figure 6. Collagen deposition measured by Sirius Red staining. Pancreatic tissue sections were prepared from the above mentioned pancreata. Sirius Red staining was performed. **(a).** Representative images of Sirius Red staining showing collagen deposition area as red. **(b).** Quantification of collagen deposition with % area/HPF (high power field) is presented as mean ± SEM from each group/sex. n=6 mice/sex in injury groups, n=4 mice/sex in recovery groups. *p<0.05 *vs.* time-matched controls. #p<0.05 *vs.* CP4W.

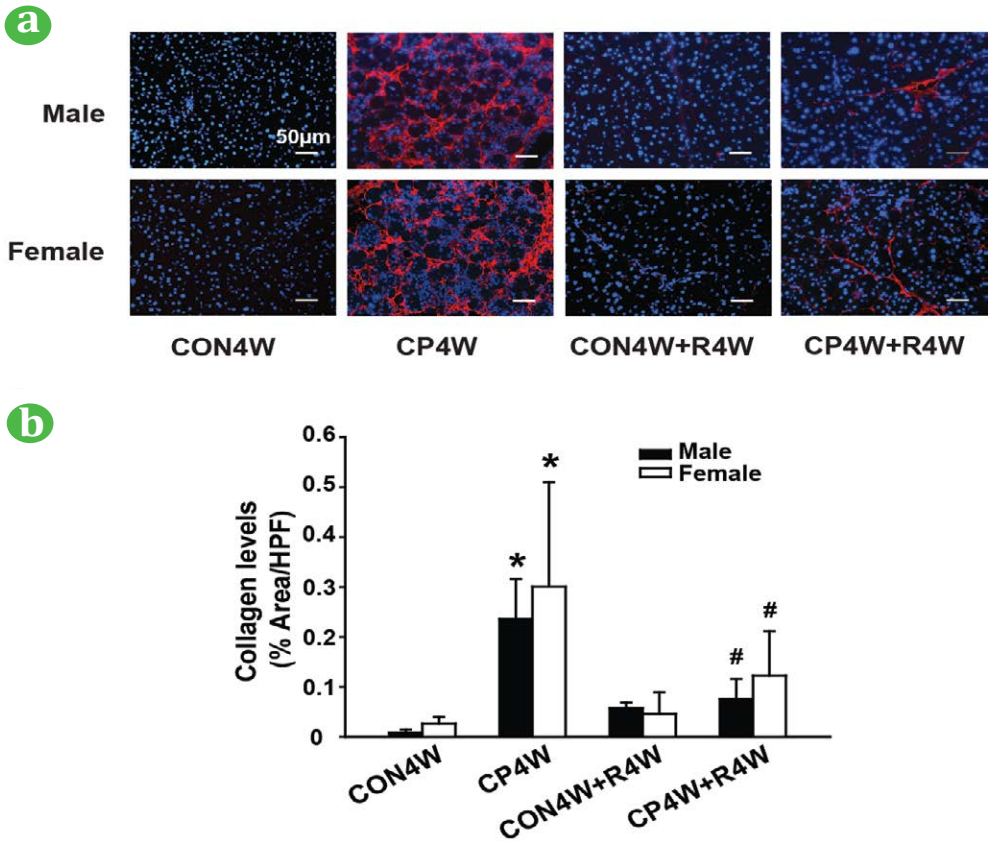

Figure 7. Col1 protein expression detected by immunofluorescence (IF) staining. Pancreatic tissue sections were prepared from the above mentioned pancreata. IF was performed using antibody against Col1. **(a).** Representative images of Col1 IF showing Col1 positive area as red, nucleus staining by DAPI as blue. **(b).** Quantification of Col1 IF with % area/HPF is presented as mean ± SEM from each group/sex. n=6 mice/sex in injury groups, n=4 mice/sex in recovery groups. *p<0.05 *vs.* time-matched controls. #p<0.05 *vs.* CP4W.

DISCUSSION

The pathophysiological study of pancreatitis largely depends on animal models due to a lack of access to human pancreatic tissue. Animal model studies, even with their limitations, continue to provide assistance in advancing our understanding of pancreatitis pathogenesis. In this study, we utilized the widely used, cerulein-induced CP model to investigate potential differences between male and female mice during CP injury and recovery. Regarding the influence of sex in CP response, we did not find differences in CP severity or recovery between male and female mice. These findings are consistent with the results from an L-arginine-induced AP model, in which both male and female C57BL/6 mice had similar levels of AP severity [35]. However, as aforementioned, in a genetically impaired autophagy-induced CP model, male mice developed more pronounced CP than females [24, 25]. In contrast, in an autoimmune pancreatitis model, female mice developed AP earlier and more frequently and severely than males [36, 37]. Finally, in a diet-induced pancreatitis model, female mice experienced more severe forms of AP than males [26, 27, 28]. These inconsistent findings show that male and female mice have variable reactions to pancreatitis depending on model, to which our study contributes by revealing no observable sex-dependent responses to CP injury and recovery in the cerulein-induced CP model. Though existing animal models have proven useful for studying the pathophysiology of pancreatitis, research concerning the influence of sex in pancreatitis may require further characterization of current models or the development of new animal models.

Our results demonstrate a recovery in CP after the cessation of cerulein injection. More specifically, cerulein-induced acinar injury is reversible, while pancreas to body weight ratio and fibrosis are only partially reversible. Other animal studies and models of pancreatitis have shown results consistent with ours. In a study with repeated lipopolysaccharide injections (LPS) for 3 weeks to induce CP in alcohol-fed rats, withdrawal of alcohol led to a resolution of pancreatic lesions due to increased pancreatic stellate cell apoptosis and a subsequent partial regression of fibrosis [33]. Another study, using a cerulein-induced AP model, demonstrated that after 7 days of recovery following AP induction, mice had a reorganization of parenchyma structure, a removal of necrotic debris, and a reversal of inflammatory infiltrates [38]. All these results confirm that withdraw of insults to the pancreas can lead to recovery of pancreatic injury.

Regarding the limitations of this study, the dosage of cerulein may be a modifiable factor in future studies. While we applied the commonly used dosage of cerulein (50 μg/kg) for an induction period of 4 weeks, it is possible that excess pancreatic injury was elicited. Therefore, we can only evaluate sex-dependent responses on CP severity, and observed no differences between male and female mice. To investigate sex-dependent responses on CP incidence, varying doses of cerulein injections may be necessary in future studies. In addition, the relative small sample size,

particularly in recovery group, is a potential limitation of our study. However, our study demonstrates a consistent pattern showing that male and female mice have similar responses to cerulein-induced CP injury and recovery. These findings, by directly comparing male and female mice in cerulein-induced CP and recovery, should provide insights for future study design and data interpretation.

The results from this cerulein-induced model study confirm the reversible nature of pancreatic injury in animals and show that male and female mice of this model have comparable responses to pancreatic injury and recovery. Our results support the use of either male or female mice of the cerulein-induced CP model for pathophysiologic studies. However, more reliable animal CP models that reflect the discrepancy of male and female influence are necessary for the investigation of sex-dependent mechanisms. Ultimately, a better understanding of the disease mechanisms may lead to the development of novel, targeted therapies for CP patients.

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Conflict of Interest

The authors declare that they have no conflicts of interest.

References

1. Etemad B, Whitcomb DC. Chronic pancreatitis: Diagnosis, classification, and new genetic developments. Gastroenterology 2001; 120:682-707. [PMID: 11179244]

2. Raimondi S, Lowenfels AB, Morselli-Labate AM, Maisonneuve P, Pezzilli R. Pancreatic cancer in chronic pancreatitis; aetiology, incidence, and early detection. Best Pract Res Clin Gastroenterol 2010; 24:349-358. [PMID: 20510834]

3. Rickels MR, Bellin M, Toledo FG, Robertson RP, Andersen DK, Chari ST, et al. Detection, evaluation and treatment of diabetes mellitus in chronic pancreatitis: Recommendations from pancreasfest 2012. Pancreatology 2013; 13:336-342. [PMID: 23890130]

4. Yadav D, Whitcomb DC. The role of alcohol and smoking in pancreatitis. Nat Rev Gastroenterol Hepatol 2010; 7:131-145. [PMID: 20125091]

5. Forsmark CE. Management of chronic pancreatitis. Gastroenterology 2013; 144:1282-1291 e1283. [PMID: 23622138]

6. Habtezion A. Inflammation in acute and chronic pancreatitis. Curr Opin Gastroenterol 2015; 31(5):395-399. [PMID: 26107390]

7. Lerch MM, Gorelick FS: Models of acute and chronic pancreatitis. Gastroenterology 2013; 144:1180-1193. [PMID: 23622127]

8. Zhan X, Wang F, Bi Y, Ji B. Animal models of gastrointestinal and liver diseases. Animal models of acute and chronic pancreatitis. Am J Physiol Gastrointest Liver Physiol 2016; 311:G343-355. [PMID: 27418683]

9. Ji B, Bi Y, Simeone D, Mortensen RM, Logsdon CD. Human pancreatic acinar cells lack functional responses to cholecystokinin and gastrin. Gastroenterology 2001; 121:1380-1390. [PMID: 11729117]

10. Murphy JA, Criddle DN, Sherwood M, Chvanov M, Mukherjee R, McLaughlin E, et al. Direct activation of cytosolic ca2+ signaling and enzyme secretion by cholecystokinin in human pancreatic acinar cells. Gastroenterology 2008; 135:632-641. [PMID: 18555802]

11. Saluja A, Logsdon C, Garg P. Direct versus indirect action of cholecystokinin on human pancreatic acinar cells: Is it time for a judgment after a century of trial? Gastroenterology 2008; 135:357-360. [PMID: 18616945]

12. Liang T, Dolai S, Xie L, Winter E, Orabi AI, Karimian N, et al. Ex vivo human pancreatic slice preparations offer a valuable model for studying pancreatic exocrine biology. J Biol Chem 2017; 292:5957-5969. [PMID: 28242761]

13. Saito YA, Schoenfeld P, Locke GR, 3rd. The epidemiology of irritable bowel syndrome in north america: A systematic review. Am J Gastroenterol 2002; 97:1910-1915. [PMID: 12190153]

14. Center MM, Jemal A, Ward E. International trends in colorectal cancer incidence rates. Cancer Epidemiol Biomarkers Prev 2009; 18:1688-1694. [PMID: 19505900]

15. Cavallini G, Frulloni L, Pederzoli P, Talamini G, Bovo P, Bassi C, et al: Long-term follow-up of patients with chronic pancreatitis in italy. Scand J Gastroenterol 1998; 33:880-889. [PMID: 9754738]

16. Frulloni L, Gabbrielli A, Pezzilli R, Zerbi A, Cavestro GM, Marotta F, et al. Chronic pancreatitis: Report from a multicenter italian survey (pancroinfaisp) on 893 patients. Dig Liver Dis 2009; 41:311-317. [PMID: 19097829]

17. Yadav D, Timmons L, Benson JT, Dierkhising RA, Chari ST. Incidence, prevalence, and survival of chronic pancreatitis: A population-based study. Am J Gastroenterol 2011; 106:2192-2199. [PMID: 21946280]

18. Hirota M, Shimosegawa T, Masamune A, Kikuta K, Kume K, Hamada S, et al. The sixth nationwide epidemiological survey of chronic pancreatitis in japan. Pancreatology 2012; 12:79-84. [PMID: 22487515]

19. Wilcox CM, Sandhu BS, Singh V, Gelrud A, Abberbock JN, Sherman S, et al. Racial differences in the clinical profile, causes, and outcome of chronic pancreatitis. Am J Gastroenterol 2016; 111:1488-1496. [PMID: 27527745]

20. Yadav D, Lowenfels AB. The epidemiology of pancreatitis and pancreatic cancer. Gastroenterology 2013; 144:1252-1261. [PMID: 23622135]

21. Yadav D, Hawes RH, Brand RE, Anderson MA, Money ME, Banks PA, et al. Alcohol consumption, cigarette smoking, and the risk of recurrent acute and chronic pancreatitis. Arch Intern Med 2009; 169:1035-1045. [PMID: 19506173]

22. Lankisch PG, Lowenfels AB, Maisonneuve P. What is the risk of alcoholic pancreatitis in heavy drinkers? Pancreas 2002; 25:411-412. [PMID: 12409838]

23. Yadav D, Eigenbrodt ML, Briggs MJ, Williams DK, Wiseman EJ. Pancreatitis: Prevalence and risk factors among male veterans in a detoxification program. Pancreas 2007; 34:390-398. [PMID: 17446836]

24. Diakopoulos KN, Lesina M, Wormann S, Song L, Aichler M, Schild L, et al. Impaired autophagy induces chronic atrophic pancreatitis in mice via sex- and nutrition-dependent processes. Gastroenterology 2015; 148:626-638 e617. [PMID: 25497209]

25. Gukovsky I, Gukovskaya AS. Impaired autophagy triggers chronic pancreatitis: Lessons from pancreas-specific atg5 knockout mice. Gastroenterology 2015; 148:501-505. [PMID: 25613315]

26. Lombardi B, Estes LW, Longnecker DS. Acute hemorrhagic pancreatitis (massive necrosis) with fat necrosis induced in mice by dl-ethionine fed with a choline-deficient diet. Am J Pathol 1975; 79:465-480. [PMID: 1094837]

27. Ida S, Ohmuraya M, Hirota M, Ozaki N, Hiramatsu S, Uehara H, et al. Chronic pancreatitis in mice by treatment with choline-deficient ethionine-supplemented diet. Exp Anim 2010; 59:421-429. [PMID: 20660988]

28. Rao KN, Eagon PK, Okamura K, Van Thiel DH, Gavaler JS, Kelly RH, et al. Acute hemorrhagic pancreatic necrosis in mice. Induction in male mice treated with estradiol. Am J Pathol 1982; 109:8-14. [PMID: 6181693]

29. Gao X, Cao Y, Staloch DA, Gonzales MA, Aronson JF, Chao C, et al. Bone morphogenetic protein signaling protects against cerulein-induced pancreatic fibrosis. PLoS One 2014; 92:e89114. [PMID: 24586530]

30. Gao X, Cao Y, Yang W, Duan C, Aronson JF, Rastellini C, et al. Bmp2 inhibits tgf-beta-induced pancreatic stellate cell activation and extracellular matrix formation. Am J Physiol Gastrointest Liver Physiol 2013; 304:G804-813. [PMID: 23429583]

31. Staloch D, Gao X, Liu K, Xu M, Feng X, Aronson JF, et al. Gremlin is a key pro-fibrogenic factor in chronic pancreatitis. J Mol Med (Berl) 2015. [PMID: 26141517]

32. Perides G, Tao X, West N, Sharma A, Steer ML. A mouse model of ethanol dependent pancreatic fibrosis. Gut 2005; 54:1461-1467. [PMID: 15870229]

33. Vonlaufen A, Phillips PA, Xu Z, Zhang X, Yang L, Pirola RC, et al. Withdrawal of alcohol promotes regression while continued alcohol intake promotes persistence of lps-induced pancreatic injury in alcohol-fed rats. Gut 2011; 60:238-246. [PMID: 20870739]

34. Demols A, Van Laethem JL, Quertinmont E, Degraef C, Delhaye M, Geerts A, et al. Endogenous interleukin-10 modulates fibrosis and regeneration in experimental chronic pancreatitis. Am J Physiol Gastrointest Liver Physiol 2002; 282:G1105-1112. [PMID: 12016137]

35. Kui B, Balla Z, Vasas B, Vegh ET, Pallagi P, Kormanyos ES, et al. New insights into the methodology of l-arginine-induced acute pancreatitis. PLoS One 2015; 10:e0117588. [PMID: 25688985]

36. Kanno H, Nose M, Itoh J, Taniguchi Y, Kyogoku M. Spontaneous development of pancreatitis in the mrl/mp strain of mice in autoimmune mechanism. Clin Exp Immunol 1992; 89:68-73. [PMID: 1352748]

37. Qu WM, Miyazaki T, Terada M, Okada K, Mori S, Kanno H, et al. A novel autoimmune pancreatitis model in mrl mice treated with polyinosinic:Polycytidylic acid. Clin Exp Immunol 2002; 129:27-34. [PMID: 12100019]

38. Lugea A, Nan L, French SW, Bezerra JA, Gukovskaya AS, Pandol SJ. Pancreas recovery following cerulein-induced pancreatitis is impaired in plasminogen-deficient mice. Gastroenterology 2006; 131:885-899. [PMID: 16952557]