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Short Communication

Pathological analysis of lesions in the exocrine pancreas of rats induced by Zinc Maltol

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Abstract: The pancreas plays an important role in the homeostasis of zinc (Zn), a nutritionally essential metal. In several previous studies, Zn ions induced inflammatory changes in the exocrine pancreas; however, little is known about Zn complexes. In this study, we microscopically, immunohistochemically, and ultrastructurally examined pancreatic lesions in Sprague-Dawley (SD) rats induced by a 4-week repeated oral dose toxicity study of Zinc Maltol (ZM), a zinc (II) complex. ZM induces acinar atrophy and increases the number of duct-like structures. Immunohistochemistry revealed a decrease in the number of trypsin-positive cells, and an increase in the number of SOX9-positive cells. Interstitial fibrosis and macrophage infiltration also correlated with the degree of acinar atrophy. Electron microscopic evaluation revealed that the acinar cells that lost granules were surrounded by fibroblasts and collagen fibers. In conclusion, we provided a detailed description of ZM-induced pancreatic lesions in SD rats. (DOI: 10.1293/tox.2023-0063; J Toxicol Pathol 2023; 36: 205–211)

Key words: zinc overload, zinc complex, metal toxicity, pancreatitis, histopathological evaluation, transmission electron microscope evaluation

Zinc (Zn) is a nutritionally essential metal for retaining protein structure and function as a component of transcription factors and approximately 2,000 enzymes, which are involved in growth and development, the immune system, DNA synthesis and RNA transcription, utilize zinc as a co-factor[1]. Although some literature reviews have demonstrated that Zn ion overload induces inflammatory changes in the exocrine pancreas of rodents, the detailed histological features have not been well documented[2–5]. Zinc Maltol (ZM) is a zinc (II) complex that has been reported to possess insulinomimetic activity; however, little data are available regarding its toxicity compared to other zinc complexes[6, 7]. Therefore, we evaluated ZM toxicity in Sprague-Dawley (SD) rats by oral gavage once daily for 4 weeks. The major manifestations of ZM-related toxicity in SD rats are anemia and histopathological changes in the pancreas, femur, and stomach as we previously reported[8]. Pancreatitis-like lesions were induced in the pancreas of all rats at a dose of 1,000 mg/kg/day. To further elucidate ZM-induced pancreatic lesions, we performed immunohistochemical staining and transmission electron microscopy (TEM) to scrutinize the histological features.

This study was conducted in accordance with the Guidelines for Animal Care and Use at Otsuka Pharmaceutical Co., Ltd. and approved by the Institutional Animal Care and Use Committee of the testing facility. Five-week-old male Sprague-Dawley rats ([Crl:CD [SD]], specific pathogen-free [SPF]) were obtained from Charles River Japan Inc. (Shiga, Japan). The rats were allotted on the basis of body weight to the control group (ZM 0 mg/kg/day) or the ZM 1,000 mg/kg/day group (five rats each). Each group was orally administered ZM, synthesized in house, by gavage once daily for 4 weeks, with 0.5% methyl cellulose solution as the vehicle. All the rats were anesthetized with isoflurane and euthanized by exsanguination. Further details regarding the animal experiments are described in Hitomi M, *et al*[8]. Major organs including the pancreas were fixed with 10% neutral buffered formalin and embedded in paraffin. All paraffin-embedded pancreatic tissues of the control group and ZM 1,000 mg/kg/day group were stained with hematoxylin and eosin (HE) and Masson's trichrome for microscopic evaluation. For immunohistochemical analysis, pancreatic tissue sections were deparaffinized and hydrated. After antigen retrieval and endogenous peroxidase inhibition, the sections were blocked with 10% goat serum and incubated with primary antibodies at room temperature (RT) for 60 min, followed by incubation with secondary antibodies at RT for 30 min. Positive reactions were visualized by 3,3′-di-

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aminobenzidine (DAB) plus Chromogen Solution (Dako, Glostrup, Denmark) followed by nuclear counterstaining using hematoxylin. The following antibodies were used to detect cells at the pancreatic lesions: trypsin as an acinar marker, SOX9 as a ductal marker, vimentin as a pan-mesenchymal cell marker, α-SMA and desmin as myofibroblast markers, GFAP as a pancreatic stellate cell (PSC) marker, Iba-1 as a macrophage marker, CD3 as a T-cell marker, and CD20 as a B-cell marker. The staining conditions are listed in Table 1. For TEM examination, pancreatic tissue samples were obtained from one control rat and one ZM-treated rat with severe lesions, as evaluated by microscopy. Tissue samples were pre-fixed in a mixture of 2.5% glutaraldehyde and 2% paraformaldehyde, post-fixed in 1% osmium tetroxide, stained en bloc with uranyl acetate, dehydrated with a graded series of acetone, and embedded in epoxy resin. The prepared tissue samples were sectioned ultra-thinly using an ultramicrotome, stained with lead citrate, and observed with a TEM (JEM-1400 plus; JEOL, Tokyo, Japan).

The grading of the microscopic evaluations is presented in Table 2. No remarkable changes were observed in the islets of any of the rats. In the exocrine pancreas, minimal to severe atrophy of acinar cells, with an increase in duct-like structures, was observed in all ZM-treated rats (Fig. 1B, 1C). The affected acini occasionally contained fewer granules in the cytoplasm and a dilated lumen. Infiltration of mononuclear cells and increased fibrous tissue were observed in the stroma surrounding the atrophied acinar and duct-like structures. Stained with Masson's trichrome, an increase in the light blue extracellular matrix, considered to be collagen fibers, was observed in the ZM-treated rats (Fig. 1E, 1F). Increased mitotic figures and single-cell necrosis of acinar cells were also observed in almost all ZM-treated rats. No significant changes were detected in the pancreases of the control rats.

The results of immunohistochemistry are presented in Table 3 and Fig. 2. In contrast to control rats (Fig. 2A), the number of trypsin-positive acini was reduced in ZM-treated rats graded as moderate, and only a few acini were positive in ZM-treated rats graded as severe (Fig. 2B, 2C). Dilated or atrophied acinar cells were negative or slightly positive for trypsin in the ZM-treated rats. SOX9 was positive in the round nuclei of the ductal epithelium and small, oval nuclei scattered around the acini (centroacinar cells and intercalated ducts) in all rats (Fig. 2D–2F). In addition, the nuclei of duct-like structures and dilated acini were positive for SOX9 in ZM-treated rats (Fig. 2E, 2F). The number and size of centroacinar cells and intercalated ducts increased in comparison with control rats. Notably, SOX9-positive cells were conspicuous in severe lesions and occasionally aggregated to form duct-like clusters.

The number of vimentin-positive cells increased with the severity of acinar atrophy. Almost all cells were positive, except for the remaining acini and duct-like structures in

Table 1. The Details of Condition for Immunohistochemical Stain

1st antibody				Antigen retrieval	2nd antibody
Antigen [Clone]	Supplier	Catalog No.	Dilution		
SOX9 [EPR14335]	Abcam, Cambridge, UK	ab185230	×2,000	98°C, 15 min in EDTA buffer (pH8.0)	Histofine Simple Stain Rat MAX-PO (R) (Nichirei Biosciences, Tokyo, Japan)
αSMA[1A4]	Dako	M0851	×1,000	98°C, 15 min in Citrate buffer (pH6.0)	
CD20	Thermo, Waltham, MA, USA	RB-9013	×100		
Trypsin	LS Bio, Seattle WA, USA	LS-B17021	×500		
Iba-1	Fujifilm Wako Osaka, Japan	019-1974	×2,000	-	
CD3 [F7.2.38]	Dako	M7254	×1,000	98°C, 15 min in EDTA buffer (pH8.0)	Histofine Simple Stain Rat MAX-PO (M) (Nichirei)
Vimentin [V9]	Dako	M0725	×200	98°C, 15 min in Citrate buffer (pH6.0)	
GFAP	BD PharMingen, Franklin Lakes, NJ, USA	556329	×1,000		
Desmin [D33]	Abcam,	ab8470	×100	-	

Table 2. Microscopic Evaluation of the Pancreases of ZM-treated Rats

Group		ZM 1,000 mg/kg				
Number of animals		5				
Findings	Grade	-	±	+	++	+++
Atrophy, acinar cell, diffuse		0	2	1	1	1
Fibrosis, interstitium		2	1	1	1	0
Infiltration, mononuclear cell, interstitium		1	2	1	1	0
Increase, mitotic figure, acinar cell		0	2	3	0	0
Single cell necrosis, acinar cell		1	3	1	0	0

Grade: -, none; ± , minimal; +, mild; ++, moderate; +++, severe. ZM: Zinc Maltol.

severe lesions (Fig. 3A, 3B). Interstitial spindle cells surrounding the duct-like structure were positive for α-SMA and desmin, and increased in correlation with the severity of acinar atrophy (Fig. 3C–3F). GFAP was less positive for spindle cells in all rats (Fig. 3G, 3H). Most interstitial infiltrating cells were positive for Iba-1 in ZM-treated rats with severe lesions (Fig. 3I, 3J). Furthermore, positive cells for CD3 and CD20 were infrequently observed (Fig. 3K–3N).

In the TEM evaluation, atrophic acinar cells observed in ZM-treated rats with severe lesions were encompassed by fibroblasts and collagen fibers and incorporated a few secretory granules. The cytoplasm of the acinar cells was

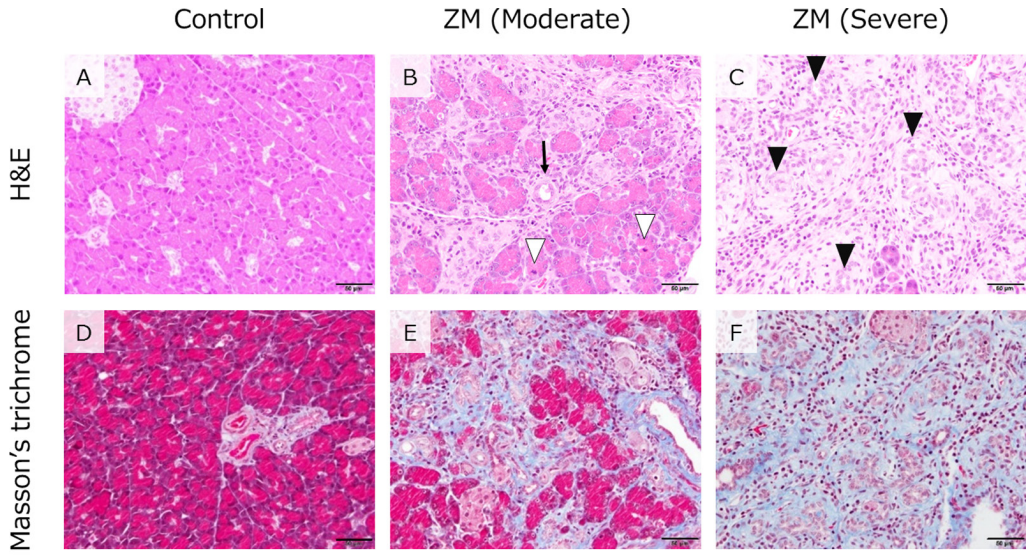

Fig. 1. Light microscopy images of the pancreas stained with hematoxylin and eosin (HE: A–C) and Masson's trichrome (D–F). (A, D) Control rats. (B, E) ZM-treated rats with moderate acinar atrophy with decreased granules and a dilated lumen (black arrow); mitotic figures were increased (white arrowhead). (C and F) ZM-treated rats with severe acinar atrophy. Almost all the acini were lost and replaced by duct-like structures (black arrowhead). (D–F) Increased fibrous extracellular matrix was observed in ZM-treated rats. Bars represent 50 μm. ZM: Zinc Maltol.

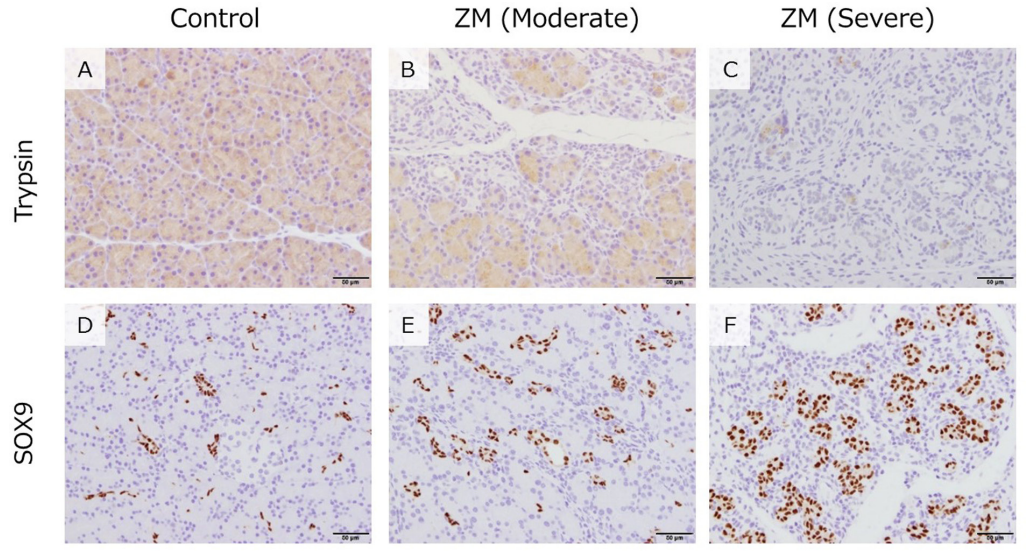

Fig. 2. Immunohistochemical analysis for the pancreas in (A, D) control rats, (B, E) ZM-treated rats with moderate lesions, and (C, F) ZM-treated rats with severe lesions. (A) The acini in the control were diffusely positive for trypsin. (B) The number of trypsin-positive acini were reduced in moderate lesions. (C) Only a few acini were slightly positive in severe lesions. Compared with the (D) control, SOX9-positive nuclei increased in (E) moderate to (F) severe lesions. Centroacinar cells and intercalated ducts were observed as scattered small cells positive for SOX9 around acini in the control and increased in proportion to the severity of acinar atrophy in ZM-treated rats. They sometimes organized into duct-like clusters in severe lesions. Bars represent 50 μm. ZM: Zinc Maltol.

Table 3. Grading of Immunohistochemistry on Pancreas of ZM-treated Rats

Group		ZM 1,000 mg/kg				
Number of animals		5				
Antibody	Grade	-	±	+	++	+++
Trypsin		0	2	1	1	1
SOX9		0	1	1	2	1
Vimentin		0	1	1	2	1
α-SMA		0	2	1	2	0
Desmin		0	2	2	0	1
GFAP		4	0	1	0	0
Iba-1		0	1	1	2	1
CD3		3	0	2	0	0
CD20		5	0	0	0	0

Grade: -, no positive cells; ± , few positive cells; +, a small number of positive cells; ++, a moderate number of positive cells; +++, a large number of positive cells. Trypsin was +++, SOX9, Vimentin, α-SMA and Desmin were ± and GFAP, Iba-1, CD3 and CD20 were - in 2 control animals. ZM: Zinc Maltol.

characterized by focal degeneration surrounded by a limiting membrane, and contained various structures with heterogeneous densities (Fig. 4A–4D). No PSCs characteristically containing lipid droplets were detected.

Histopathologically, ZM manifests as diffuse acinar atrophy in the exocrine pancreas, whereas the islet cells are less affected. The pancreatic lesions vary from mild acinar atrophy without inflammation to severe acinar atrophy accompanied by fibrosis and mononuclear cell infiltration, which is described as stereotypical chronic pancreatitis. Acinar atrophy and increased duct-like structures were similar to those observed in a previous 13-week toxicity study of zinc sulfate[5].

Chronic pancreatitis is defined as the progressive chronic inflammation of the pancreatic tissues[9]. It is characterized by persistent inflammation that results in the loss of endocrine and exocrine compartments owing to atrophy and/or replacement with fibrotic tissue. Related to chronic pancreatitis, a damaged pancreas sometimes shows epithelial structures termed “acinar-ductal metaplasia” (ADM),

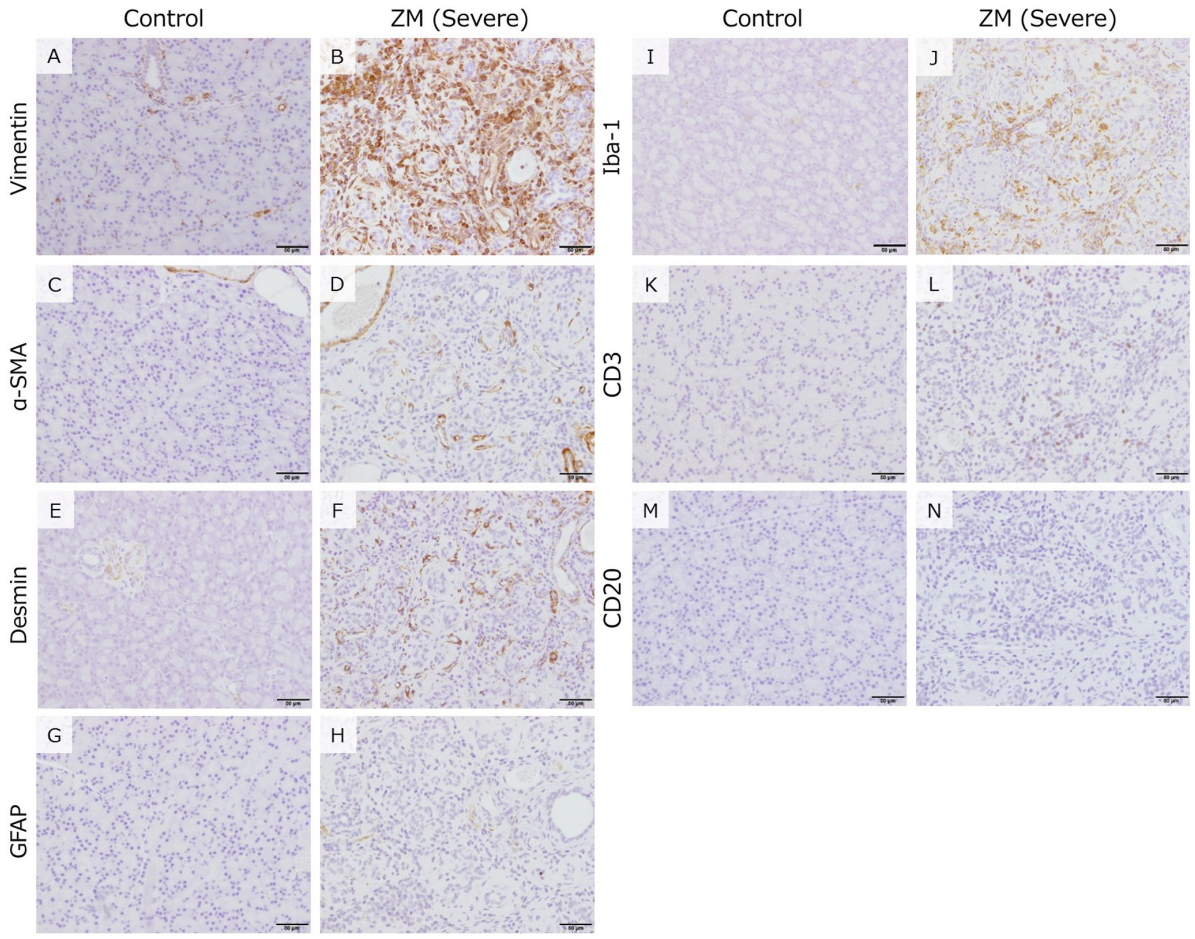

Fig. 3. Immunohistochemical analysis for interstitial components of the pancreas in (A, C, E, G, I, K, M) control rats and (B, D, F, H, J, L, N) ZM-treated rats with severe lesions. (A, B) Vimentin-positive cells were sporadically observed in interstitium in the control rats and increased in ZM-treated rats. (C–F) Spindle cells positive for α-SMA and desmin were occasionally surrounding the duct-like structures in ZM-treated rats. (G, H) GFAP was rarely positive in both control and ZM-treated rats. (I, J) Most of the infiltrating cells were positive for Iba-1 in ZM-treated rats, (K–N) while only a few cells were positive for CD3 and CD20. Bars represent 50 μm. ZM: Zinc Maltol.

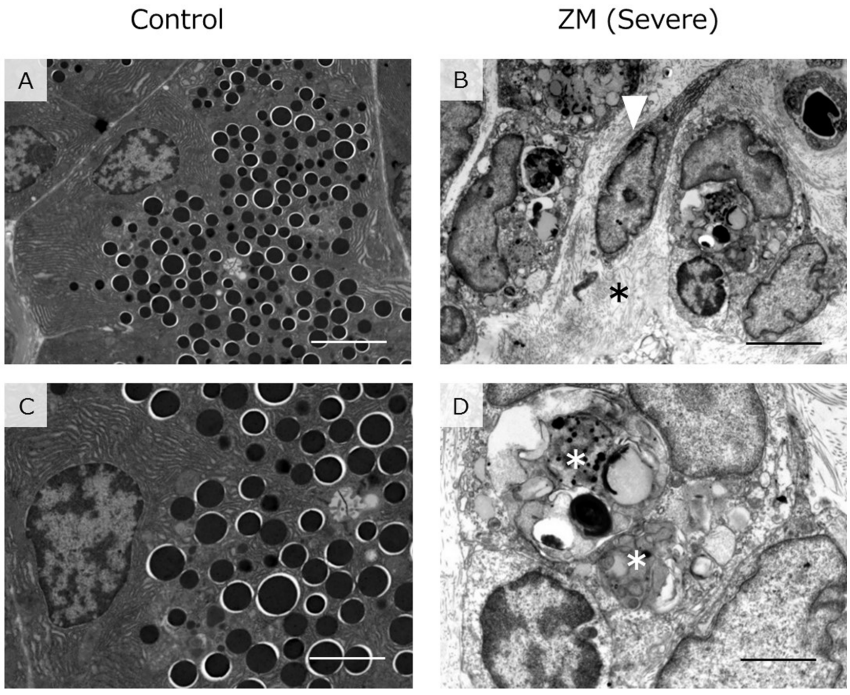

Fig. 4. Transmission electron microscopic evaluation on the pancreases of (A, C) control and (B, D) ZM-treated rats. (A, B) Original magnification ×2,000. Bars represent 5 μm. (C, D) A higher magnification of A and B. Original magnification ×5,000. Bars represent 2 μm. (B) Atrophied acini surrounded by fibroblasts (white arrowhead) and collagen fibers (black asterisk) were observed in ZM-treated rats. (D) The acini featured by a lack of secretory granules and focal cytoplasmic degeneration surrounded by a limiting membrane and containing various structures with heterogeneous density (white asterisk). ZM: Zinc Maltol.

which represents acinar cells that have dedifferentiated to duct-like structures[10–13]. ADM is a characteristic of chronic pancreatitis; however, its underlying mechanism and details remain controversial[10].

The pancreatic lesions described in this study were characterized by various grades of acinar atrophy. The atrophy and decreased numbers of acini and trypsin-positive cells observed in ZM-treated rats were confirmed by histopathological analysis. Conversely, the staining grade of the nuclei positive for SOX9, the ductal fate determinant, increased with the severity of acinar atrophy. Centroacinar cells, which line the intraacinar ducts, connect the acini to the intercalated ducts[14]. Centroacinar cells and intercalated ducts were observed as SOX9-positive cells with small, oval nuclei scattered around the acini in the control pancreas. SOX9-positive cells sporadically aggregated into duct-like clusters, particularly in severe lesions. Several studies have shown that centroacinar cells are multipotent progenitors in pancreatic regeneration because of their rapid proliferation following acinar injury, such as through partial pancreatectomy or cerulein-induced pancreatitis[14, 15]. Thus, increased numbers of centroacinar cells and intercalated ducts may replace degenerated acinar cells and differentiate into mature ducts, resulting in ADM in ZM-induced lesions. Similar features, degeneration and a decrease in acini, ADM, and clarification of centroacinar cells have also been reported in mice and rats fed a diet containing 30,000 ppm zinc sulfate for 13 weeks[5]. The atrophied acini with decreased granules and dilated lumina were slightly positive for trypsin, whereas their nuclei were positive for SOX9, suggesting they have features of both acinar and ductal cells. Morphological identification through TEM evaluation between acinar cell without granules and ductal cells (including centroacinar cells and intercalated ducts) was not characterized in the present study. Moreover, the origin and differentiation have not been clearly confirmed, and further investigation is required to understand the nature of SOX9-positive acinar cells.

PSCs, which are interstitial resident cells in the exocrine pancreas, have received attention for their assumed contribution to fibrosis by switching to active myofibroblasts producing abundant collagen[10, 16–19]. GFAP, a PSC marker, was less positive and the characteristics of PSCs could not be detected by TEM in any sample in this study. Contrary to the absence of PSCs, the positive cells for α-SMA and desmin, a myofibroblast and proto-myofibroblast marker, respectively, increased in proportion to the severity of interstitial fibrosis. Therefore, these fibrotic lesions may be predominantly induced by migrating fibroblasts and myofibroblasts.

Inflammatory cell infiltration is less severe compared with severe acinar atrophy and fibrosis. Most of the infiltrating cells were positive for Iba-1. In contrast, only a few cells tested positive for CD3 and CD20, lymphocyte markers. Despite the fact that mild to moderate lymphocytic infiltration and acinar apoptosis were reported in the 13-week repeated dose toxicity study of zinc oxide nanoparticles at a dose of 536.8 mg/kg/day[4], the pancreatic lesions induced by

4-week repeated doses of 1,000 mg/kg/day ZM showed the pathological characteristics of a later stage.

A literature review of a Zn-ion dose study showed acinar injury or degeneration; however, it did not illustrate the detailed mechanism of Zn-induced inflammation or acinar injury[20]. The exocrine pancreas is considered vulnerable to Zn overload because pancreatic acinar cells contain high levels of metallothionein, a family of intracellular nonspecific metal-binding proteins that contribute to Zn homeostasis[3, 20, 21]. Excessive Zn is absorbed into the body, accumulates in the exocrine pancreas, and causes intracellular injury by generating oxidative stress[3, 22]. Many reports suggest that injured acini play a major role in the pathogenesis of pancreatitis by promoting autodigestion, atrophy, necrosis, and inflammation through the leakage of enzymes such as trypsin[10]. Infiltrating macrophages that remain in the lesion release cytokines, followed by the activation of fibroblasts, resulting in fibrosis[10]. Taking the above into consideration, inflammation and fibrosis may have occurred secondary to intracellular injury in acinar cells, because remarkable acinar atrophy and regeneration were observed even in individuals with slight inflammation.

In this study, we demonstrated that repeated doses of ZM induces chronic pancreatitis-like lesions in the exocrine pancreas. Additionally, we describe novel detailed histopathological findings and compare them with previously reported Zn ion-induced lesions. The ZM-induced pancreatic lesions were considered comparable to those reported for Zn ion-induced lesions. In summary, ZM-induced findings in the pancreas ranged from minimal to severe, and the lesions showed acinar atrophy accompanied by an increase in duct-like structures. Considering the literature, it is likely that excessive ingestion of Zn initially induces acinar atrophy, which leads to chronic pancreatitis. In conclusion, our detailed histopathological description of ZM-induced lesions is invaluable and provides useful information for the toxicity assessment of Zn compounds.

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References

1. Read SA, Obeid S, Ahlenstiel C, and Ahlenstiel G. The role of zinc in antiviral immunity. Adv Nutr. **10**: 696–710. 2019. [Medline] [CrossRef]
2. Onosaka S, Tetsuchikawahara N, and Min KS. Paradigm shift in zinc: metal pathology. Tohoku J Exp Med. **196**: 1–7. 2002. [Medline] [CrossRef]
3. Tetsuchikawahara N, Min KS, and Onosaka S. Attenuation of zinc-induced acute pancreatitis by zinc pretreatment: dependence on induction of metallothionein synthesis. J Health Sci. **51**: 379–384. 2005. [CrossRef]
4. Seok SH, Cho WS, Park JS, Na Y, Jang A, Kim H, Cho Y, Kim T, You JR, Ko S, Kang BC, Lee JK, Jeong J, and Che JH. Rat pancreatitis produced by 13-week administration of zinc oxide nanoparticles: biopersistence of nanoparticles and possible solutions. J Appl Toxicol. **33**: 1089–1096. 2013. [Medline] [CrossRef]
5. Maita K, Hirano M, Mitsumori K, Takahashi K, and Shirasu Y. Subacute toxicity studies with zinc sulfate in mice and rats. J Pestic Sci. **6**: 327–336. 1981. [CrossRef]
6. Adachi Y, Yoshida J, Kodera Y, Kato A, Yoshikawa Y, Kojima Y, and Sakurai H. A new insulin-mimetic bis(allixinato) zinc(II) complex: structure-activity relationship of zinc(II) complexes. J Biol Inorg Chem. **9**: 885–893. 2004. [Medline] [CrossRef]
7. Yoshikawa Y, Ueda E, Kawabe K, Miyake H, Sakurai H, and Kojima Y. New insulin-mimetic zinc (II) complexes; bis-maltolato zinc(II) and bis-2-hydroxypyridine-N-oxido zinc(II) with Zn(O4) coordination mode. Chem Lett. **29**: 874–875. 2000. [CrossRef]
8. Hitomi M, Akizawa F, Kondo S, Dogishi K, Fujiwara S, Kimoto H, and Moroki T. Four-week repeated oral dose toxicity study of zinc maltol in rats. Food Chem Toxicol. **175**: 113755. 2023. [Medline] [CrossRef]
9. Kleeff J, Whitcomb DC, Shimosegawa T, Esposito I, Lerch MM, Gress T, Mayerle J, Drewes AM, Rebours V, Akisik F, Muñoz JED, and Neoptolemos JP. Chronic pancreatitis. Nat Rev Dis Primers. **3**: 17060. 2017. [Medline] [CrossRef]
10. Murtaugh LC, and Keefe MD. Regeneration and repair of the exocrine pancreas. Annu Rev Physiol. **77**: 229–249. 2015. [Medline] [CrossRef]
11. Greaves P. Liver and pancreas. In: Histopathology of Preclinical Toxicity Studies: Interpretation and Relevance in Drug Safety Evaluation, 4th ed. Academic Press, New York. 433–535. 2012.
12. Kopp JL, von Figura G, Mayes E, Liu FF, Dubois CL, Morris JP 4th, Pan FC, Akiyama H, Wright CV, Jensen K, Hebrok M, and Sander M. Identification of Sox9-dependent acinar-to-ductal reprogramming as the principal mechanism for initiation of pancreatic ductal adenocarcinoma. Cancer Cell. **22**: 737–750. 2012. [Medline] [CrossRef]
13. Tokoro T, Tezel E, Nagasaka T, Kaneko T, and Nakao A. Differentiation of acinar cells into acinoductular cells in regenerating rat pancreas. Pancreatology. **3**: 487–496. 2003. [Medline] [CrossRef]
14. Beer RL, Parsons MJ, and Rovira M. Centroacinar cells: at the center of pancreas regeneration. Dev Biol. **413**: 8–15. 2016. [Medline] [CrossRef]
15. Cleveland MH, Sawyer JM, Afelik S, Jensen J, and Leach SD. Exocrine ontogenies: on the development of pancreatic acinar, ductal and centroacinar cells. Semin Cell Dev Biol. **23**: 711–719. 2012. [Medline] [CrossRef]
16. Garcia PE, Scales MK, Allen BL, and Pasca di Magliano M. Pancreatic fibroblast heterogeneity: from development to cancer. Cells. **9**: 2464. 2020. [Medline] [CrossRef]
17. Desmoulière A, Darby IA, and Gabbiani G. Normal and pathologic soft tissue remodeling: role of the myofibroblast, with special emphasis on liver and kidney fibrosis. Lab Invest. **83**: 1689–1707. 2003. [Medline] [CrossRef]
18. Hashimoto A, Karim MR, Izawa T, Kuwamura M, and Yamate J. Immunophenotypical analysis of pancreatic intersti-

tial cells in the developing rat pancreas and myofibroblasts in the fibrotic pancreas in dogs and cats. J Vet Med Sci. **79**: 1920–1926. 2017. [Medline] [CrossRef]
19. Omary MB, Lugea A, Lowe AW, and Pandol SJ. The pancreatic stellate cell: a star on the rise in pancreatic diseases. J Clin Invest. **117**: 50–59. 2007. [Medline] [CrossRef]
20. Minami T, Shimane M, Tanaka H, Namikawa K, and Ichida S. Pancreatic exocrine damage induced by subcutaneous injection of a low dosage of zinc. Biol Trace Elem Res. **84**: 169–179. 2001. [Medline] [CrossRef]
21. De Lisle RC, Sarras MP Jr, Hidalgo J, and Andrews GK. Metallothionein is a component of exocrine pancreas secretion: implications for zinc homeostasis. Am J Physiol. **271**: C1103–C1110. 1996. [Medline] [CrossRef]
22. Taneja SK, and Mandal R. Attenuation of Zn-induced acute pancreatitis in Wistar rat fed on Cu- and Mg-enriched modified poultry egg Psi. Biol Trace Elem Res. **125**: 245–254. 2008. [Medline] [CrossRef]