EFFECTS OF FLUORIDE ON PANCREATIC DIGESTIVE ENZYME ACTIVITIES AND ULTRASTRUCTURE IN YOUNG PIGS

Xiu-an Zhan,[a] Jian-xin Li,[a] Zi-rong Xu,[a] Min Wang[a]

Hangzhou, China

SUMMARY: This study was conducted to investigate the effects of fluoride on pancreatic digestive enzyme activities and ultrastructure in young pigs. Three groups of crossbred barrows were exposed to 100, 250, and 400 mg F^-/kg (from NaF) in their diets for 50 days. Compared to a control group, the activities of pancreatic lipase and protease (but not amylase) were significantly decreased. Pancreatic acinar cells showed markedly swollen mitochondria and loss of mitochondrial cristae. Endoplasmic reticulum (ER) was markedly dilated and its folds were irregular. These results indicate that excessive fluoride in the diet can inhibit pancreatic digestive enzyme activities and cause ultrastructural changes, which may lead to a series of biochemical and pathological abnormalities.

Keywords: Digestive enzymes; Endoplasmic reticulum; Lipase; Mitochondria; Pancreatic acinar cells; Protease; Young pigs.

INTRODUCTION

Prevalent in many parts of the world, chronic fluorosis is caused by excessive ingestion of fluoride over a prolonged period and endangers the health of humans as well as animals. In addition to its well-known effects on the skeleton and on teeth, fluoride can exert toxic effects on many other tissues and organs, giving rise to a broad array of symptoms and pathological changes.[1] Recently, we found that fluoride induced excessive production of nitric oxide and reactive oxygen species, enhanced lipid peroxidation, and disturbed the antioxidant system of young pigs; therefore oxidative damage from oxidative stress might be an important pathway for fluoride toxicity in soft tissues.[2] Owing to fluoride in environmental pollution and in mineral supplements such as calcium phosphate and limestone, chronic fluorosis in domestic animals is an increasingly serious problem resulting in considerable economic losses in animal production.

Although fluorosis has been investigated for many years, there are relatively few studies of its effect on the digestive system such as the pancreas, a small organ located near the lower part of the stomach and the beginning of the small intestine. Enzyme secretions of the exocrine pancreas are required for hydrolysis of nutrients present in food and feed.[3] The present study was undertaken to assess the effects of fluoride on pancreatic digestive enzyme activities and on ultrastructure in the pancreas.

MATERIALS AND METHODS

Animals and experimental diets: Thirty-two 50-day-old barrows with an average body weight of about 17 kg were acclimatized for one week and then

[a]Feed Science Institute, College of Animal Science, Zhejiang University, No.268 Kaixuan Road, Hangzhou, China, 310029.
For correspondence: Jian-xin Li, E-mail:doubleten@zju.edu.cn.

allotted randomly to four groups of eight. These animals were the same pigs we used earlier,[2] which were fed a basal diet containing 6.2 mg F^-/kg in the control group 1, supplemented by 100, 250, and 400 mg F^-/kg diet from NaF in experimental groups 2, 3, and 4, respectively. On the 50th day of the feeding trial, all pigs were deprived of feed for 12 hr and then slaughtered under general anesthesia using halothane.

Pancreatic digestive enzyme activities: The pancreas from the slaughtered pigs was homogenized and centrifuged. The supernatant was saved for determining the activities of lipase (EC 3.1.1.3), protease, and amylase (EC 3.2.1.1). Lipase was determined at 37°C by a pH-stat titration using tributyrin as substrate according to the method of Erlanson-Albertsson et al.[4] Protease activity was analyzed with the modified method of Lynn and Clevette-Radford[5] using azocasein as substrate. One lipase or protease unit is defined as the amount of enzyme that hydrolyses 1 μmol of substrate per minute. Amylase was determined by the iodometric method.[6] One amylase unit is the amount of enzyme that hydrolyses 10 mg of starch in 30 min.

Transmission electron microscopy: The pancreas was excised and fixed with 2.5% glutaraldehyde in 0.1 M phosphate buffer (pH 7.4). Each sample was dehydrated through graded ethanol solution and then embedded in epoxy resin. Ultra-thin sections were cut and stained with uranyl acetate and lead citrate. The sections were examined under a Jeol JEM-1230 transmission electron microscope.

Statistical analysis: The significance of the difference between means was determined by analysis of variance (ANOVA) with $p<0.05$ being considered significant.

RESULTS

Compared with the control group 1, the activity of pancreatic lipase in pig groups 2, 3, and 4 exhibited a significant monotonic decrease, whereas the activity of pancreatic protease decreased more slowly and was significantly lower in group 4. Pancreatic amylase activity, on the other hand, showed a small, non-significant paradoxical increase in group 3 before declining again in group 4 (Table).

Table. Effects of fluoride on pancreatic digestive enzyme activities in young pigs[a,b]

Group[c]	1	2	3	4
Lipase	450.20±59.31	345.93±70.76*	333.92±66.83*	355.91±63.06*
Protease	88.04±13.28	80.46±7.76	81.90±14.47	72.54±9.09*
Amylase	293.22±19.85	291.62±23.09	304.75±30.57	285.96±48.59

[a]Values are mean±SD; n=8 per group. Compared with the control group, *p<0.01.
[b]Pancreatic digestive enzyme activities are expressed as U/g pancreas.
[c]Group 1 was the control, and the other three groups were the experimental ones with 100, 250, and 400 mg F^-/kg diet (from NaF).

In the fluoride-treated groups, pancreatic acinar (secretory) cells showed marked swollen mitochondria and loss of mitochondrial cristae. The degree of mitochondrial swelling and cristae loss increased with the dose of fluoride in the diet. The mitochondrial matrix was of low electron density and appeared transparent in groups 2–4 (Figure 1). In some areas, the outer and inner mitochondrial membranes were damaged (Figures 1B and 1C), and small vacuoles appeared in the cytoplasm (Figure 1C).

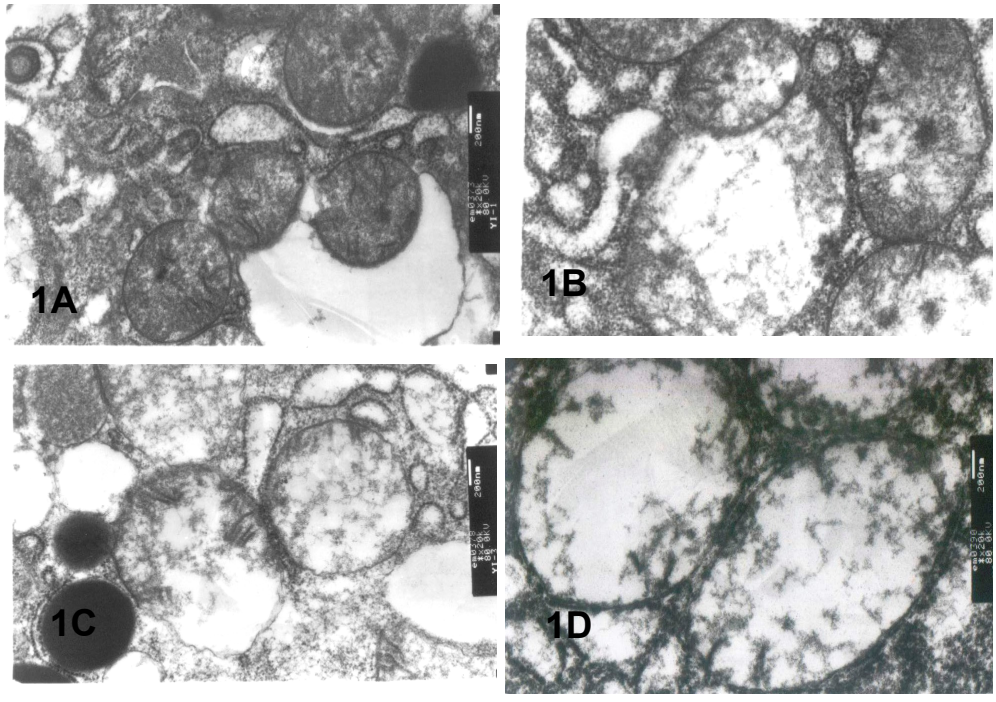

Figure 1. Transmission electron micrographs of mitochondria in pancreas. A, B, C, and D are representative micrographs of groups 1, 2, 3, and 4, respectively (x20,000).

Endoplasmic reticulum (ER) was markedly dilated and its folding pattern was irregular in group 2 (Figure 2B, next page). Some of the ER were ruptured and their cisternae were significantly enlarged (Figures 2C and 2D, next page).

DISCUSSION

This study revealed that excessive fluoride inhibits the activities of pancreatic lipase and protease and causes observable ultrastructural changes. These effects might be an important reason for growth depression induced by fluorosis. Excessive production of free radicals induced by fluoride may damage the structures of digestive enzymes and reduce their activities.[2,7]

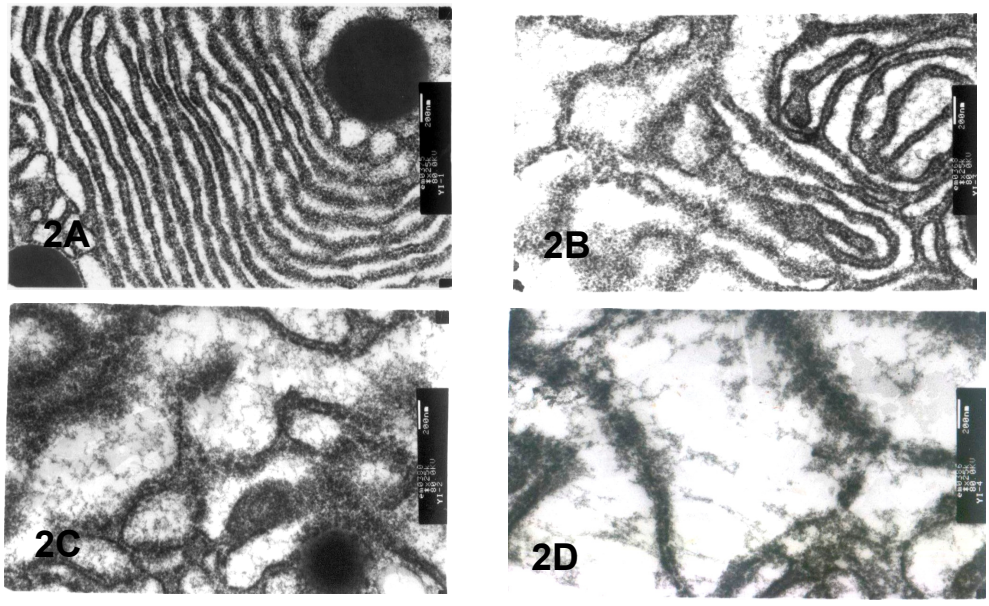

Figure 2. Transmission electron micrographs of endoplasmic reticulum in pancreas. A, B, C, and D are representative micrographs of groups 1, 2, 3, and 4 respectively, (x25,000).

The ultrastructural changes in the pancreas observed here are in accordance with previous findings in thyroid, liver, and kidney of fluorotic animals.[8,9] The mitochondrion is an organelle that is the site of aerobic cellular respiration and is responsible for the conversion of food and feed to usable energy.[10] Its morphological changes would disturb the metabolism of substances in the mitochondria and suppress ATP (adenosine triphosphate) synthesis. Enlargement of mitochondria in fluoride-treated pigs is believed to a compensatory process due to ATP deficiency.[8] Endoplasmic reticulum (ER) is a membrane network within the cytoplasm of cells that is involved in the synthesis, modification, and transport of cellular materials. The dilation of ER in fluoride-treated pigs indicates enhancement of detoxification reactions and its rupture would suppress protein synthesis that might reduce the pancreatic secretions, including digestive enzymes. The destruction of mitochondria and ER can be attributed to oxidative stress induced by fluoride, which can seriously damage the structure of cells and organelles, especially their membranes.[2,7]

In conclusion, we have found that excessive fluoride in the diet can inhibit pancreatic digestive enzyme activities and damage pancreatic ultrastructure, especially mitochondria and ER, which may in turn lead to a series of biochemical and pathological abnormalities.

ACKNOWLEDGEMENTS

This research was supported by the National Basic Research Program of China (Project 2004CB117505). We also wish to thank Wei-fen Li for her skillful technical assistance.

REFERENCES

1 Waldbott GL. Nonskeletal fluorosis [editorial review]. Fluoride 1978;11:111-4.
2 Zhan XA, Xu ZR, Li JX, Wang M. Effects of fluorosis on lipid peroxidation and antioxidant systems in young pigs. Fluoride 2005;38:157-61.
3 Rinderknecht H. Pancreatic secretory enzymes. In: Go VLW, DiMagno JD, Gardner E, Lebenthal E, Reber HA, Scheele GA, editors. The Pancreas: biology, pathobiology and disease. New York: Raven Press; 1993. p. 219-51.
4 Erlanson-Albertsson C, Larsson A, Duan R. Secretion of pancreatic lipase and colipase from rat pancreas. Pancreas 1987;2:531-5.
5 Lynn KR, Clevette-Radford NA. Purification and characterization of hevin, a serin protease from Hevea brazilliensis. Biochemistry 1984;23:963-4.
6 Harms DR, Camfield RN. An automated iodometric method for the determination of amylase. Am J Med Technol 1966;32:341-7.
7 Liu G, Chai C, Cui L. Fluoride causing abnormally elevated serum nitric oxide levels in chicks. Environ Toxicol Pharmacol 2003;13:199-204.
8 Zhan CW, Huo DJ. Ultrastructural findings in liver, kidneys, thyroid gland and cardiac muscle of rabbits following sodium fluoride administration. Fluoride 1988;21:32-8.
9 Liu GY, Chai CY, Kang SL. Effects of fluoride on ultrastructure of thyroid in chicks. Chin J Vet Sci 2002;22:512-4 (in Chinese).
10 Enger ED, Ross FC. Concepts in biology. Cell structure and function. 10th ed. Beijing: Science Press; 2003. p. 58-83.

Published by the International Society for Fluoride Research
http://homepages.ihug.co.nz/~spittle/fluoride-journal.htm
Editorial Office: 727 Brighton Road, Ocean View, Dunedin 9051, New Zealand