The Effect of Zinc Therapy on Lysosomal Inclusion Bodies in Intestinal Epithelial Cells in Acrodermatitis Enteropathica

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Summary

Two patients with acrodermatitis enteropathica had abnormal lysosomal inclusion bodies in the intestinal epithelial cells while in relapse but fewer smaller ones after zinc therapy. The third patient with acrodermatitis enteropathica in remission on zinc therapy had no inclusion bodies. The smaller inclusion bodies were similar to those found in coeliac disease.

Abbreviation

AE, acrodermatitis enteropathica

Lysosomal inclusion bodies have been described in the apical cytoplasm of small intestinal epithelial cells in β lipoprotein deficiency (6), coeliac disease (14), tropical sprue (12), infantile malnutrition (5), Crohn's disease (15), and after aspirin ingestion (9). It has been suggested that these inclusion bodies are autophagosomes, bacteria or dead epithelial cells ingested by their neighbours.

Previous electron microscopic studies of the mucosa in acrodermatitis enteropathica (AE) have demonstrated cytoplasmic vesicles and dilated intercellular spaces in the epithelial cells (13), abnormal Paneth cells (11) and both abnormal Paneth cells and multivesicular bodies in the enterocyte (3, 4).

This paper describes very large lysosomal inclusion bodies found in two patients with AE and the effect of zinc therapy on these bodies.

MATERIALS AND METHODS

Three patients with AE were studied. Biopsies were taken under fluoroscopic control just distal to the Ligament of Trietz using a two-port biopsy capsule. One biopsy was used for an investigation of the [⁶⁵Zn] uptake by the mucosa, and this work is reported elsewhere (1). The tissue:medium concentration ratios, which are a measure of the mucosal uptake of zinc, are included in Table 1, which gives details of the patients and therapy. These biopsies were compared with a total of 52 jejunal biopsies taken for diagnostic purposes (44 children and eight adults) with normal histology and ultrastructure and a total of 15 jejunal biopsies (eight children and seven adults) in which a diagnosis of coeliac disease was made on clinical history and histologic and ultrastructural findings. All biopsies were fixed in 2.5% glutaraldehyde in phosphate buffer pH 7.4, post-fixed in 1% OsO₄, dehydrated and embedded in epon or araldite. Semithin and ultrathin sections were cut using an LKB ultramicrotome and glass knives. The semithin sections were mounted on glass slides, stained with toluidine blue and observed in a light microscope. The ultrathin sections were transferred to copper/nickel grids, double stained with a saturated solution of uranyl acetate in 50% ethanol and Reynold's lead citrate and observed in a Philips 300 transmission electron microscope.

RESULTS

All the patients described as histologically normal at the light microscope level had normal ultrastructure (16). In the AE patients

Table 1. Clinical and biochemical details of the three patients with acrodermatitis enteropathica studied

Patient	Age	Therapy ¹ (mg ZnSO ₄)	Plasma zinc ² (μmole/liter)	Plasma ² alkaline phosphatase (i.u./liter)	T:M ratio ³	Clinical features
1 F	9y 3mo	None	3.7	43	1.55	Depressed mood; anorexic; oral, facial, and acral dermatitis; hyperkeratosis on thighs
	9y 6mo	220 mg (three times a day)	14.9	140	1.67	Asymptomatic, normal skin
2 F	5y 0mo	110 mg (twice a day)	11.4	287	1.53	Asymptomatic
	5y 3mo	None	8.6	101	1.81	Depressed; cheilitis; nasolabial and retroauricular eczematous plaques
3 M	4y 1mo	100 mg (twice a day)	15.8	132	1.96	Asymptomatic

¹ 220 mg ZnSO₄ contains 50 mg (0.76 mmole) elemental zinc.

² Laboratory reference ranges: plasma zinc, 11-24 μmole/liter (72-157 μg/100 ml). Age and sex related alkaline phosphatase: patient 1, 78-185 i.u./liter and patients 2 and 3, 71-177 i.u./liter.

³ Control values (n = 11) 4.3-11.0 (mean ± S.E. 7.5 ± 0.8).

abnormal inclusion bodies and secretory granules similar to those described previously (11) were present in some Paneth cells. The enterocytes of Patient 3 (on zinc therapy) had normal ultrastructure; however, very large abnormal lysosomal inclusion bodies were found in the apical cytoplasm of some enterocytes in AE patients 1 and 2. These inclusion bodies were not found on all villi and when present could be found in any region of a villus. On some villi, groups of inclusion body-containing enterocytes were present and inclusion body-containing enterocytes were occasionally found along the whole length of a villus.

In the first biopsy of Patient 1 (AE in relapse), the inclusion bodies were generally round in shape, contained floccular electron dense material and were 1.5–3.5 μm in diameter (Fig. 1). Other smaller heteromorphous inclusion bodies were also present and these were found together with the larger bodies in the same enterocyte but generally in a more apical position in the cytoplasm. In the second biopsy of Patient 1 (AE in remission), the inclusion bodies were different from those described above. They were generally round in shape and varied from 0.5–2.0 μm in diameter. Each inclusion body contained numerous pleomorphic electron dense granules, which were surrounded by a halo of electron-lucent material and the background substance was very electron dense (Fig. 2). The inclusion body granules were very similar in ultrastructure to the goblet cell granules found in this biopsy (Fig. 3).

In Patient 2, lysosomal inclusion bodies were only found in the second biopsy (AE in relapse) and were smaller and fewer in number than those found in Patient 1. The inclusion bodies were round in shape and contained remnants of degenerating cellular organelles such as mitochondria (Fig. 4). The inclusion bodies were found together with smaller electron dense bodies similar to the lysosomes found in normal biopsies. In this biopsy the majority of enterocytes contained numerous small electron dense lysosomes.

Pleomorphic lysosomal inclusion bodies were seen on numerous occasions in the villous enterocytes of some patients with coeliac disease (children and adults). The inclusion bodies varied from 0.5–1.8 μm and contained electron dense material together with structures resembling cellular organelles (Fig. 5). Some of the inclusion bodies were very similar in morphology to those seen in AE and inclusion bodies containing structures resembling those found in degenerating goblet cells were often found (Fig. 6).

Similar inclusion bodies to those seen in AE or coeliac disease were never seen in normal biopsies.

DISCUSSION

Lysosomal inclusion bodies do occur in small numbers in the intestinal epithelial cells of normal subjects but large numbers are only found in disease states. This is confirmed by our observation that one patient with AE had no inclusion bodies while in remission after zinc therapy, but several small ones were observed in relapse. The other patient with AE in relapse with a very low plasma zinc had many large inclusion bodies but fewer smaller ones after the commencement of zinc therapy. The third AE patient who was in remission on zinc therapy had no inclusion bodies. These inclusion bodies are not specific to AE as we have observed them in coeliac disease, confirming other workers' observations (14).

Fig. 2. Villous enterocytes with large granule containing inclusion bodies (arrowheads). Note electron density of granules and their surrounding electron lucent halos. A.E. Patient 1 (in remission). Jejunum $\times 8800$.

Fig. 1. Villous enterocytes containing large inclusion bodies with a floccular electron dense composition (arrowheads) and small heteromorphous inclusion bodies (arrows). A.E. Patient 1 (in relapse). Jejunum $\times 8800$.

Fig. 3. Goblet cell with secretory granules of similar composition to those seen in the inclusion bodies of Figure 2. A.E. Patient 1 (in remission). Jejunum $\times 8800$.

Fig. 4. Villous enterocyte with a large inclusion body containing remnants of mitochondria (arrowheads). Numerous lysosomes (arrows) are also present. A.E. Patient 2 (in relapse). Jejunum $\times 8800$.

Fig. 5. Villous enterocytes with numerous pleomorphic inclusion bodies (arrowheads). Note structures resembling degenerating mitochondria (arrows) in the inclusion bodies and infiltrating lymphocytes (L). Adult Coeliac Disease. Jejunum $\times 7300$.

In both AE and in coeliac disease these inclusion bodies appeared to contain remnants of goblet cells including large mucin masses. They were much larger and morphologically unlike the multivesicular bodies described by Braun *et al.* (3). They were only present when the patient's plasma zinc was low and signs of zinc deficiency were evident.

Fig. 6. Villous enterocytes with inclusion bodies (arrowheads) of similar composition to an adjacent degenerating goblet cell (g). The inclusion bodies are very similar to the large floccular bodies seen in Figure 1. Adult Coeliac Disease. Jejunum $\times 5900$.

Zinc deficiency has been reported in coeliac disease (7), the other condition in which the abnormal inclusion bodies were found. There is no evidence at present that zinc deficiency alone causes goblet cell abnormalities although abnormal Paneth cells are found in AE (11) which can return to normal after zinc therapy (2).

Abnormal Paneth cells were found in these three patients with AE and it is possible that zinc is essential for normal secretory granule formation, stabilization and release in both Paneth and goblet cells. Abnormal pancreatic acinar cells have been reported in zinc deficient rats (10) so zinc may be necessary for normal secretion in all exocrine and endocrine cells.

It is suggested that some of the large abnormal lysosomal inclusion bodies found in both AE and in coeliac disease are derived from phagocytosis of damaged goblet cells by adjacent epithelial cells (8) and that the goblet cell abnormalities are due to zinc deficiency.

Patients with AE on therapy who have minimal structural abnormalities of the intestinal mucosa still have abnormal mucosal zinc uptake as shown by the tissue:medium concentrations, which have not been significantly improved by the return to normal appearance.

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 17. This study was approved by the Standing Committee on Ethical Practice of the Hospital for Sick Children, Great Ormond Street, London, and informed consent was obtained from the parents of the children. Informed consent was obtained from either the patients or their legal guardians for diagnostic biopsy.
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