SHORT COMMUNICATION

A defect in zinc uptake by jejunal biopsies in acrodermatitis enteropathica

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Summary

1. In a system in vitro, $^{65}$Zn accumulation by jejunal mucosal biopsies from patients with acrodermatitis enteropathica was found to be markedly reduced compared with controls.

2. We suggest that defective uptake of zinc by enterocytes is the primary abnormality responsible for the zinc deficiency underlying this disorder.

Key words: acrodermatitis enteropathica, jejunum, zinc absorption.

Introduction

Acrodermatitis enteropathica is a rare inherited disorder, transmitted by an autosomal recessive gene, which, if untreated, is normally fatal in early childhood. Management of the disease was radically transformed by the discovery that all the clinical manifestations are the consequence of zinc deficiency (Moynahan & Barnes, 1973), and can be reversed by the administration of oral zinc supplements (Moynahan 1974). These findings added a powerful impetus to studies concerned with the role of zinc and other trace elements in human metabolism, particularly in disease states. Zinc is widely distributed in biological tissues and is essential for optimum activity of a variety of important enzymes, including alkaline phosphatase, carbonic anhydrase, DNA polymerase and thymidine kinase.

The mechanism by which children with acrodermatitis enteropathica become zinc deficient has not been clearly defined, though Lombeck, Schnippering, Ritzl, Feinendegen & Bremer (1975) reported reduced whole-body retention of orally administered $^{65}$Zn, suggesting that intestinal absorption of zinc may be defective.

We investigated this possibility further by studying the uptake in vitro of labelled zinc by jejunal biopsies taken from patients with acrodermatitis enteropathica.

Methods

Three patients with acrodermatitis enteropathica have been studied: two were girls, aged 4 and 9 years (patients 1 and 2), and the third was a boy, aged 4 years (patient 3). In all three the diagnosis of acrodermatitis enteropathica had been made during the first 6 months of life on the basis of typical clinical features and a prompt response to di-iodohydroxyquinoline (Diodoquin). This was subsequently discontinued in favour of oral zinc supplements, which had been the only treatment in all three for at least 2 years before this study. All three patients were studied whilst on zinc therapy, and the two girls were re-studied when the typical manifestations of acrodermatitis enteropathica recurred after treatment had been withdrawn. This occurred after 2 weeks in one case and after 3 months in the other. The 12 control subjects (age
range 4 months–12 years, mean 5 years) comprised children with suspected malabsorption who were proven to be normal after extensive investigation.

Approval for the study was obtained from the Hospital's Standing Committee on Ethical Practice.

Biopsies were taken under fluoroscopic control from a point immediately distal to the duodeno-jejunal flexure, using a modified two-port Kugler–Crosby capsule as described by Kilby (1976). One biopsy specimen was prepared for examination by light microscopy and the other was used for 65Zn uptake studies with a method in vitro adapted from that described by Crane & Mandelstam (1960). Each biopsy was immediately placed in oxygenated Krebs-Henseleit buffer (pH 7.3) at room temperature. In the laboratory, the biopsy was incubated in 5 ml of continuously oxygenated Krebs-Henseleit buffer (pH 7.3, osmolarity 285 mosmol/l) at 37°C. The buffers were modified by adjustment of the ionized calcium content to 1.27 mmol/l. The incubation medium contained 2.5 mmol of glucose/l as a metabolizable substrate, incorporating a tracer of [14C]glucose as an indicator of tissue viability. The medium also contained zinc chloride in a concentration of 5–10 μmol/l, incorporating a tracer of [65Zn]zinc chloride.

After exactly 10 min incubation, the tissue was removed, blotted and weighed, before being solubilized in Soluene 300 (Packard Instrument Co., Downers Grove, Ill. 60515, U.S.A.) and counting for radioactivity in both beta and gamma counters. Aliquots of the incubation medium were similarly counted, and their zinc content was measured by atomic absorption spectroscopy. The tissue to medium concentration gradients for 65Zn and [14C]glucose were calculated, and represent the ratio of the final concentration of each isotope in the intracellular fluid to its final concentration in the incubation medium. The presence of two isotopes in the incubation media used in these experiments precluded the accurate counting of a third in the form of a radioactively labelled extracellular space marker. However, an extracellular space of approximately 24% of the wet tissue weight was previously determined for similar jejunal biopsy material by the use of [hydroxy-14C]methylthiulin.

Results

The morphological appearance of all biopsies was normal by light microscopy. All biopsies were of similar weight.

The mean tissue/medium gradient of 65Zn for biopsies from the 12 control subjects was 7.54 (SEM ± 0.83), compared with 1.70 ± 0.18 for the three patients with acrodermatitis enteropathica (see Fig. 1); this difference is statistically highly significant (t-test, P < 0.001). No appreciable difference was demonstrated between values obtained with and without zinc supplementation in the two patients in whom this effect was measured.

The mean tissue/medium gradient of [14C]glucose for all biopsies was 32.49 ± 10.40; tissue viability was demonstrated in every biopsy.

Discussion

The data presented in this paper demonstrate the existence of a marked defect of mucosal zinc uptake in the small intestine of patients with acrodermatitis enteropathica. Such a defect has not previously been reported.

Diarrhoea is a characteristic feature of untreated acrodermatitis enteropathica, and non-specific histological abnormalities, including partial villous atrophy, have been demonstrated in jejunal biopsies in several cases (Rayhanzadeh & Dantzig, 1974; Kelly, Davidson, Townley & Campbell, 1976). Such histological findings are rare, and were absent in our patients. These changes appear to be the result of zinc deficiency, since they are not found in treated cases (Kelly et al., 1976). It could be argued that reduced zinc uptake by small-intestinal biopsies in untreated acrodermatitis
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enteropathica might occur as a consequence of non-specific malabsorption induced by zinc deficiency. However, we have shown that impairment of mucosal zinc uptake occurs to the same degree when patients are zinc repleted and asymptomatic as when they are zinc deficient and symptomatic.

It is currently believed that intestinal zinc absorption occurs in a number of steps, the zinc atom being passed on by a series of binding ligands present in the gut lumen, the enterocyte brush border, cytosol, basolateral membrane and, finally, the plasma (Evans, 1976). The system in vitro we have described would be able to detect dysfunction at the brush border and in the cytosol, but not at the other sites. A brush-border carrier has not been identified, but the presence of such a carrier is suggested by the finding of Davies (1972), with loops of rat duodenum in situ, that zinc transport from the lumen displayed saturation kinetics. Hahn & Evans (1973) and Hurley and her colleagues (Hurley, Duncan, Sloan & Eckhert, 1977) have demonstrated the presence of a low-molecular-weight zinc-binding complex within the cytosol of small-intestinal enterocytes from rats killed 1 h after intragastric administration of $^{65}$Zn. Song & Adham (1978) have published data which suggest that the functions of mucosal carrier and cytosolic ligand may in fact be accomplished by a single molecular species in the rat small-intestinal enterocyte. Our findings would be consistent with a genetically determined absence, or structural abnormality, of any one of these zinc-binding molecules in acrodermatitis enteropathica.

Oral zinc therapy is effective in acrodermatitis enteropathica (Moynahan, 1974). We do not believe that this is inconsistent with the presence of a specific defect of intestinal zinc absorption in this condition. Presumably, the presence of large quantities of zinc in the intestinal lumen is able to overcome the defect, either because the normal mechanism for zinc absorption is not totally inoperative, or because alternative mechanisms for zinc entry are available.

Acknowledgments

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References