Oral zinc supplements in non-responsive coeliac syndrome: effect on jejunal morphology, enterocyte production, and brush border disaccharidase activities

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SUMMARY Three patients with clinically mild non-responsive adult coeliac syndrome were treated for eight weeks with oral zinc sulphate, and detailed biochemical and morphological studies were performed on the jejunal mucosa before and on treatment. Plasma zinc levels were reduced before treatment and rose to normal levels with therapy; mucosal zinc was normal before treatment and increased after therapy. Oral zinc supplementation did not alter the villous morphology, intra-epithelial lymphocyte count or *in vitro* enterocyte production rate. In addition, there was no improvement in the clinical status of the patients. There was, however, a small increase in the activity of certain of the brush border disaccharidases. This effect may be due to direct stabilisation of the brush border membrane. The clinical value of zinc supplements in coeliac syndrome remains to be determined.

In patients with typical coeliac syndrome gluten withdrawal results both in improved absorption of dietary nutrients and in regeneration of the villi of the jejunal mucosa. Non-responsive coeliac syndrome is a related disorder in which there is malabsorption and a flat jejunal mucosa with failure to regenerate villi after careful and prolonged gluten withdrawal. It has been estimated that this group of patients comprises between 10 and 20% of adult coeliacs and, in some, the malabsorption may be severe enough to cause death.¹

We have previously shown that in non-responsive coeliac syndrome, in contrast with gluten-sensitive enteropathy, there is a reduced rate of *in vitro* enterocyte production² and differences in the activities of endoplasmic reticulum and brush border enzymes of the enterocytes.³ Love and co-workers reported low serum zinc levels in six severely ill non-responsive coeliacs and obtained a clinical response after zinc supplementation.^{4,5} The aim of the present study was to determine the extent of tissue zinc

deficiency in three patients with non-responsive coeliac syndrome and to determine the effect of oral zinc supplementation on clinical status, jejunal morphology, *in vitro* enterocyte production, and the activities of brush border maltase, sucrase, and lactase.

Methods

PATIENTS

Three patients with clinically mild non-responsive coeliac syndrome were studied (Table). Non-responsive coeliac syndrome is defined as a condition with idiopathic subtotal villus atrophy and malabsorption in patients who show no morphological response to at least five years of a strict gluten free diet. All patients had malabsorption of at least two dietary nutrients and a persistently flat jejunal mucosa despite strict and prolonged gluten withdrawal confirmed both by dietary history and normal serum anti-gliadin antibody titres. The patients were clinically only mildly affected and had not received corticosteroids before the present study. Subsequently they received a course of prednisolone, 20 mg daily, after which all patients showed a significant clinical improvement with a marked increase in intestinal function and morphological appearance.

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Table Clinical details of patients with non-responsive coeliac syndrome

Patient	Age (yr)	Sex	Height (cm)	Weight (kg)	Clinical details	Antigliadin antibody titre	Time on GFD (yr)	Serum folate (ng/l)		Serum albumin (g/l)	
								Before zinc therapy	After zinc therapy	Before zinc therapy	After zinc therapy
FC	57	M	152	46	Previous malabsorption with SVA, responding clinically and histologically to GFD. Relapse after gluten ingestion with persistent SVA despite GFD. Minimal malabsorption. Faecal fat 10.2 mmol/24h. Xylose excn. 2.5 g in 5 h (25 g oral dose)	1/4	11	3.0	3.2	45	42
WW	58	M	175	60	Coeliac syndrome diagnosed aged 40 yr with symptoms from adolescence. No response to GFD with SVA, cryoglobulinaemia and focal glomerulitis. Moderate malabsorption. Faecal fat 83 mmol/24h. Xylose excn. 1.5 g in 5 h (25 g dose)	1/8	18	Receiving folic acid supplements		41	40
WS	80	F	154	52	Coeliac syndrome diagnosed aged 72 yr with symptoms for many years. Symptomatic improvement with GFD but persisting SVA. Faecal fat 37 mmol/24h. Xylose excn. 2.0 g in 5 h. (25 g dose)	1/4	8	4.4	4.6	39	40
Control values						<1/64		Range 6.0–21.0		Range 35–52	

GFD: gluten-free diet. SVA: subtotal villous atrophy.

Jejunal biopsies were obtained from each patient in the usual manner, 10 cm distal to the ligament of Treitz, with a Watson-Crosby capsule. Biopsies were divided into pieces for: (1) routine histology, measurement of surface:volume ratio⁶ and intra-epithelial lymphocyte counting⁷; (2) measurement of zinc concentration; (3) determination of *in vitro* enterocyte production²; (4) assay of maltase, sucrase, and lactase activities.⁸ Each patient continued on a gluten free diet and, in addition, received zinc sulphate capsules 220 mg three times daily for a period of eight weeks. At the end of this period samples of blood, 24 hour urine, and jejunal biopsies were collected and re-assayed.

Zinc was assayed spectrophotometrically using a Perkin Elmer atomic absorption spectrophotometer, model 360.⁹ Members of the laboratory and medical staff served as controls for plasma and urine zinc. Three patients undergoing investigation for gastrointestinal symptoms with normal jejunal morphology served as controls for measurement of surface: volume ratios. Student's paired *t* test was used for statistical analysis. The study was approved by the local ethical committee.

Results

Plasma zinc (Fig. 1) was low in all three patients, in two (WS and WW) levels were more than two

standard deviations below the mean control value. After treatment plasma zinc rose in all three patients to control values and this was statistically significant ($p < 0.01$). Twenty-four hour urine zinc did not differ from controls but rose after treatment; this rise was marked in only one patient (FC). Jejunal zinc concentration also did not differ from controls but rose in each patient after treatment; the increase was particularly marked in the same patient (FC). In none of the three patients did the period of zinc treatment result in any significant change in body weight, frequency of bowel action, haemoglobin level, plasma albumin, or serum folate.

Histological examination of the jejunal biopsies before and after treatment showed flat mucosa. Surface:volume ratios (Fig. 2) were below the control range and there was no change after zinc treatment. Intraepithelial lymphocyte counts in biopsies from the three patients were 12–36/100 epithelial cells before treatment and showed no significant change after treatment with values of 13–25/100 epithelial cells. Enterocyte production *in vitro* by jejunal mucosa (Fig. 3) did not differ from controls and did not show the increased rate characteristic of untreated gluten-responsive coeliac mucosa. After treatment with zinc there was a small, but not statistically significant, fall in *in vitro* enterocyte production.

Jejunal maltase activity (Fig. 4) in the three

Fig. 1 Plasma, urine, and jejunal zinc concentrations in control subjects and patients with non-responsive coeliac syndrome. Plasma zinc levels $\pm$ SD. Patients: $\blacktriangle$ — $\blacktriangle$, FC; $\blacksquare$ — $\blacksquare$, WW; $\bullet$ — $\bullet$, WS.

Fig. 2 Jejunal morphology in control subjects and patients with non-responsive coeliac syndrome. Symbols as in Fig. 1.

Fig. 3 In vitro jejunal enterocyte production rate in control subjects and patients with responsive or non-responsive coeliac syndrome. Symbols as in Fig. 1.

patients was low compared both with control and with untreated responsive coeliac mucosa. After zinc treatment maltase activity increased in all three patients. Jejunal sucrase activity was lower than control values and in two patients (FC and WS) lower than usually found in untreated responsive coeliac syndrome. After zinc treatment jejunal sucrase activity increased in two patients (FC and WW) and did not change in one (WS). Jejunal lactase activity (Fig. 4) was low in all three patients compared with untreated responsive coeliac mucosa. After treatment, lactase activity increased in one patient (FC) and did not change in the other two. The patient (FC) who showed the largest net increase in zinc content of plasma, 24 hour urine, and jejunal tissue also showed the greatest increase in jejunal maltase, sucrase, and lactase activities.

Discussion

The treatment of non-responsive coeliac syndrome is at present unsatisfactory; while some patients are able to maintain reasonable health on a gluten free diet with vitamin supplements, most have symptoms of moderate to severe malabsorption and in some this may be fatal.¹ The need, therefore, for a better understanding of the processes involved³ and for more effective therapy is important.

In our three patients pretreatment plasma zinc levels were low and showed a significant increase after zinc treatment. Plasma zinc is bound approximately 60% to albumin and 30% to α_2 -globulin.¹⁰ The low levels of plasma zinc are therefore not due

Fig. 4 Jejunal disaccharidase activities in control subjects and patients with responsive or non-responsive coeliac syndrome. Symbols as in Fig. 1.

to an alteration in the plasma albumin level. Low levels of plasma zinc are also found in untreated gluten-responsive coeliac syndrome.^{11,12} However, plasma zinc in one group of non-responsive coeliac patients has been found to be lower than in a group of untreated gluten-responsive patients and this also cannot be explained on lower serum albumin levels alone.⁵ Plasma zinc concentrations are, however, a poor measure of total body zinc stores¹³ and the normal level of jejunal zinc suggests that our patients may not be zinc deficient at the cellular level. The rise in plasma zinc in the three non-responders indicates that they were able to absorb significant quantities of oral zinc sulphate and it is of interest that the patient with the highest plasma zinc before and after treatment (FC) showed the greatest increase in urinary zinc. Jejunal zinc concentration in the three patients rose after zinc supplementation.

However, zinc content of leucocytes and connective tissue will contribute to the total zinc concentration in the jejunal biopsy homogenate, so that the values measured do not necessarily indicate enteroblast or enterocyte zinc content.

In none of the three patients was there a significant change in the clinical status after the period of zinc treatment. The differences between the response in the patients we have studied and that of the Belfast patients⁵ is probably because the latter group of patients were severely ill with more marked malabsorption.

Zinc treatment resulted in no subjective improvement in jejunal morphology. The failure to regenerate villi in non-responsive coeliac syndrome is associated with and probably due to a reduction in enteroblast mitotic rate.² Zinc is essential for RNA dependent DNA polymerase activity,¹⁴ so that it is reasonable to consider whether zinc deficiency might result in a reduced rate of DNA synthesis in man. Our studies, however, indicate that zinc deficiency alone is not responsible for the reduced rate of enteroblast division and enterocyte production in non-responsive coeliac syndrome.

Zinc supplementation did result in an increase in the activities of jejunal brush border disaccharidases and this may conceivably lead to improved digestive and absorptive function of the enterocyte. The mechanism of the increase in brush border enzymes are activated *in vitro* by Zn^{2+} ions^{15,16} or are themselves zinc-protein complexes.¹⁷ A more likely explanation of the beneficial effect is that Zn^{2+} ions act as membrane stabilisers^{18,19} inhibiting the degradation of the brush border enzymes.

From the clinical response obtained in the Belfast group of patients and the improvement in brush border enzyme activities that we have found, a trial of oral or intravenous zinc supplements might be of value in this syndrome, particularly in those patients who have failed to show a response to gluten withdrawal and systemic corticosteroids.

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