Eur J Pediatr (1981) 137:23–25

European Journal of
Pediatrics

The Therapeutic Effect of Amphotericin in Acrodermatitis Enteropathica: Hypothesis and Implications

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Abstract. A nine year-old girl with acrodermatitis enteropathica developed typical clinical and biochemical features of zinc deficiency on two occasions while on an oral zinc supplement. On both occasions, these features responded immediately when she was treated with amphotericin B lozenges. Studies in vitro showed that amphotericin increases the permeability to zinc of pure lipid membranes containing cholesterol. We suggest that the antibiotic enhanced zinc absorption from the oral supplement thereby effecting resolution of the patient's zinc deficiency.

Introduction

Acrodermatitis enteropathica (AE), a rare autosomal recessive condition, characterised by failure to thrive, alopecia, dermatitis and diarrhoea, is now known to be due to zinc deficiency [9] probably caused by a primary defect of zinc absorption [2, 5]. Before the discovery that zinc supplements alone could maintain a clinical remission in AE, 8-hydroxyquinoline derivatives such as 5,7 di-iodo-8-hydroxyquinoline (diiodohydroxyquinoline) were the therapy of choice [9]. The efficiency of these compounds probably derives from their ability to chelate zinc and facilitate its translocation across lipid membranes thereby enhancing zinc absorption and behaving as ionophores [1]. Initially, however, it was speculated that their effect depended upon their antifungal activity and the clinical response of a probable case of AE to an unrelated fungicide, the polyene antibiotic nystatin, supported this view [13]. This paper describes the beneficial effect of another polyene compound amphotericin B in AE, and presents an hypothesis for this effect based on liposome and partition studies.

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Methods

Blood was taken by venepuncture into trace metal free heparinised tubes; plasma zinc was determined by atomic absorption spectroscopy (AAS) (Perkin Elmer 306 spectrometer) [8]. Peroral jejunal biopsies were obtained from just distal to the ligament of Trietz under fluorescent control; a portion was sent for histology and the remainder was used for ^{65}Zn uptake studies as previously described [5]. A conventional three day trace metal metabolic balance was performed and the material was analysed as reported elsewhere [3].

Multilamellar phospholipid vesicles (liposomes) comprising phosphatidylcholine, phosphatidic acid and cholesterol in the molar ratios of 61, 5 and 33, or phosphatidylcholine and phosphatidic acid in the molar ratio of 95:5 with or without one part of amphotericin were prepared [11]. The liposomes were suspended in a solution of NaCl (94 mmol/l) and Trischloride (5 mmol/l) buffered at pH 7.5 containing ^{65}Zn labeled $ZnCl_2$ (75 μmol/l); after equilibration under N_2 excess radiotracer was removed by passage of the lipid suspensions through a sephadex G50 medium grade column (Pharmacia). Aliquots of the lipid eluate were placed in dialysis bags (Visking 8/32) and transferred to stoppered tubes containing 10 ml of an identical but nonlabeled solution. Samples of dialysate were taken at known intervals and their radioisotope content counted (LKB 1280 Ultragamma counter). The percentage of the initial count remaining within the liposomes was calculated.

The ability of amphotericin to complex zinc was investigated by studying its extraction from citrate buffered aqueous $ZnCl_2$ solution (10 μmol/l) at pHs 4, 5, 6, 7, 8, and 9 into an equal volume of a chloroform solution of amphotericin (20 μmol/l). After thorough mixing of the two phases the zinc content of the aqueous phase was determined by AAS and compared with that of control systems containing no amphotericin.

Cholesterol was purchased from Sigma and the preparation of phosphatidyl choline and phosphatidic acid is described elsewhere [6]. ^{65}Zn Cl_2 was obtained from the Radiochemical

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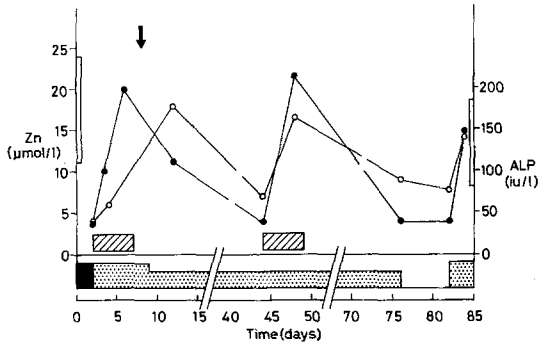

Fig. 1. Plasma zinc concentration ● and alkaline phosphatase activity ○ related to oral zinc supplementation, given as encapsulated ■ or syrup ▣ formulations, and to amphotericin administration ▨ (doses as in text). An episode of diarrhoea is indicated (↓). The areas on the ordinates indicate the laboratory reference ranges

Centre, Amersham and the amphotericin was a gift from Squibb. All other chemicals were of the purest grade supplied by British Drug Houses.

Case Report

The patient, a girl, was the second child of healthy unrelated parents. After an uneventful pregnancy she was born at term weighing 3.64 kg. She was fed cow's milk formula and developed blisters on her fingers and a perineal rash at the age of 2 weeks, and diarrhoea at one month. AE was diagnosed at 2 months and treatment with diiodohydroxyquinoline 200 mg tds resulted in a prompt remission. Oral zinc sulphate was substituted for diiodohydroxyquinoline when she was 5 years 8 months old and she remained well until 4 years later when she presented with anorexia, depression and oral and dermatological lesions characteristic of AE. Plasma zinc was 3.7 μmol/l (24.2 μg/dl) (normal range 11–24 μmol/l) and the plasma activity of the zinc metalloenzyme alkaline phosphatase (ALP) was 43 IU/l (normal range 78–185 IU/l). Jejunal biopsy histology was normal on light microscopy but the mucosal uptake of ^{65}Zn achieved a tissue-medium concentration gradient of 1.81 compared with our control values of 4.3–11.0 (mean 7.5 ± 0.8 SE). At that time she was receiving encapsulated zinc sulphate 220 mg tds providing 2.29 mmol (150 mg) of elemental zinc a day. This was changed to a syrup preparation in a similar dose and a five-day course of amphotericin lozenges (10 mg qds) was started simultaneously because her oral lesions resembled candidiasis. There was an immediate clinical and biochemical improvement (see Fig. 1). After six days she developed diarrhoea which resolved when the dose of zinc sulphate syrup was reduced to 220 mg bd [1.51 mmol (100 mg) elemental zinc].

Four weeks later her mood deteriorated and she again developed cutaneous and biochemical manifestations of zinc deficiency. There was a prompt response to a repeat course of amphotericin without any alteration in the dose of zinc sulphate. Analysis of amphotericin lozenges indicated that these would have provided only 59.1 nmol (3.87 μg) of elemental zinc daily. A month after completing the amphotericin she was completely asymptomatic though the plasma zinc concentration and ALP had fallen to 4.3 μmol/l (28 μg/dl) and 88 IU/l respectively.

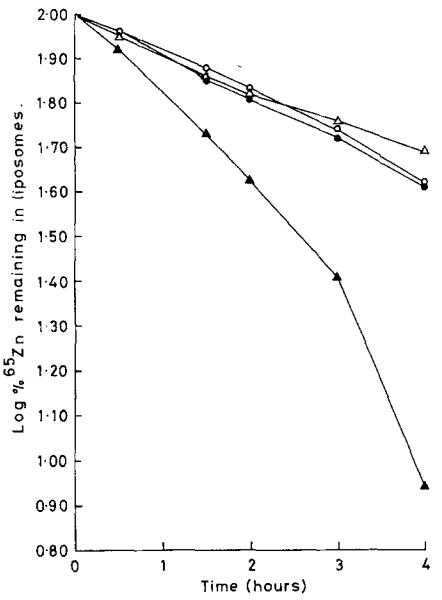

Fig. 2. The effect of incorporating amphotericin (1 μmol/100 μmol total lipid) on ^{65}Zn efflux from cholesterol containing ▲ or cholesterol free ● liposomes, compared with control preparations without amphotericin △ ○

Table 1. Oral intake, net absorption and retention of Zn, Fe, and Cu (μmol/kg/d), and of Nitrogen (gm/kg/d) in the metabolic balance study conducted while the child was on no oral zinc supplement (– indicates net intestinal secretion or loss)

	Oral intake	Net absorption	Net retention
Zinc	5.41	−1.84	−1.88
Iron	9.69	+0.39	+0.38
Copper	2.90	+0.75	+0.73
Nitrogen	0.29	+0.26	0.00

The zinc supplement was interrupted and a metabolic balance study was performed. This demonstrated net intestinal secretion and body loss of zinc in the presence of net intestinal absorption of copper, iron and nitrogen with retention of the former two elements and zero nitrogen balance (Table). After completion of the balance study skin lesions typical of AE had developed on the child's fingers and alaque nasi; an increased oral zinc sulphate supplement of 220 mg tds was introduced without recurrence of the diarrhoea and two days later the plasma zinc content and ALP had increased to 14.9 μmol/l and 140 IU/l respectively and the skin lesions had resolved. A repeat jejunal biopsy taken at that time had normal histology on light microscopy and the mucosal uptake of ^{65}Zn was still low at 1.53. Throughout this period the child's height and weight remained on the 50th and 25th percentiles respectively and no organisms were cultured from oral, skin and rectal swabs or from her faeces. The child has since remained well and thelarche commenced about six months after this study.

Partition Study

In this study, amphotericin failed to extract any zinc from the aqueous to the organic phase at any of the pHs used.

Liposome Study

As seen in Fig. 2 amphotericin had a marked effect on the permeability of the cholesterol-containing pure lipid membrances to zinc, but no effect on the liposomes from which cholesterol had been excluded.

Discussion

Apart from her earlier dependence upon diiodohydroxyquinoline therapy the diagnosis of AE in this child is substantiated by (i); the demonstration of a negative zinc balance and net intestinal secretion of zinc during the metabolic study (ii), her dependence on oral zinc supplements and (iii) the impaired mucosal uptake of ^{65}Zn in vitro.

Plasma ALP activity is a good indicator of body zinc status in experimental and clinical circumstances where zinc nutrition can be regulated [10, 12]. The association of increased plasma ALP with clinical improvement as well as increased plasma zinc concentrations in our patient is therefore indicative of an improved zinc status. Furthermore, the association of this improvement with amphotericin administeration suggests a causal realtionship although altering the zinc formulation may have contributed on the first occasion. In view of the negligible zinc content of the amphotericin lozenges, we suggest that amphotericin itself enhanced zinc absorption from the oral zinc supplement and thereby induced the clinical and biochemical remission.

The partition study showed the inability of amphotericin to form zinc complexes which would have enabled the translocation of the metal across biological membranes in a manner analogous to that proposed for diiodohydroxyquinoline [1]. However, the fungicidal properties of polyene antiobiotics, such as amphotericin, have been related to their ability to interact with membrane sterols thereby forming poorly selective transmembrane "pore units" which increase membrane permeability to water and small solutes including non electrolytes [4]. Our liposome study has shown that amphotericin increased the permeability of a pure lipid membrane to zinc and that this was indeed cholesterol dependent. To our knowledge, this is the first demonstration that these amphotericin "channels" are permeable to zinc ions, and we propose that in this patient with AE amphotericin enhanced zinc absorption from the intestinal lumen by facilitating its diffusion across lipid membranes.

The toxicity of intravenous amphotericin has been attributed to altered membrane permeability [4], but the effect of oral amphotericin on intestinal function has not been extensively studied although amphotericin has been used in vitro to increase the permeability of rabbit colon mucosal membrane to sodium and potassium [7]. This case report illustrates that such studies in relation to other cations could have important therapeutic and toxicological implications.

Acknowledgments. We thank Dr. R. S. Wells for permission to study this patient, Mr. J. Mitchell for nitrogen determinations and Allan and Hanbury and Unigate for financial assistance. PJA thanks the M.R.C. and Rank Prize Fund, DJA the M.R.C. and JMT the Cystic Fibrosis Research Trust for financial support.

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Received February 12, 1981