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THE POSSIBLE ROLE OF DIODOQUIN AS A ZINC IONOPHORE IN THE TREATMENT OF ACRODERMATITIS ENTEROPATHICA

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SUMMARY The mechanism by which 5,7-di-iodo-8-hydroxy quinoline (Diodoquin) enhances zinc absorption in the zinc deficiency disorder acrodermatitis enteropathica was investigated using liposomes. This compound increased the permeability of the pure lipid membranes to ^{65}Zn and it is proposed that the therapeutic effect of Diodoquin and related compounds may derive from their ability to act as ionophores.

INTRODUCTION Acrodermatitis enteropathica (AE), a rare autosomal recessive condition characterised by severe dermatitis, alopecia, diarrhoea and growth failure, is now known to be due to zinc deficiency (1). It was frequently fatal in childhood until it was fortuitously discovered that the antifungal agent 5,7-di-iodo-8-hydroxyquinoline (Diodoquin) could induce a complete clinical remission (2). Subsequently the successful but empirical use of this and other halogenated derivatives of 8-hydroxyquinoline such as 5-chloro 7-iodo-8-hydroxyquinoline (Enterovioform) (3) and 2-methyl,5,7-dichloro-8-hydroxyquinoline (Sterosan) (4) and 5,7-dibromo-8-hydroxy-quinoline (5) in this condition has been reported.

Abbreviations: AE Acrodermatitis enteropathica
TRIS Tris(hydroxymethyl)methylamine

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The pathophysiological importance of zinc was first appreciated when Diodoquin was found to be ineffective in a child receiving a zinc deficient synthetic diet. It has since been shown that in the untreated patient with AE, the intestine is in a net secretory state with respect to zinc and that this is reversed to net absorption either by oral Diodoquin or zinc therapy alone (6). The latter has now superceded other treatments but the pharmacological mechanism for the action of Diodoquin in AE has not been investigated.

The known chelating properties of the di-halo-8-hydroxyquinolines have been invoked to propose two possible bases for their enhancement of zinc absorption, either they chelate an abnormal intestinal polypeptide which would otherwise bind zinc intraluminally and prevent its absorption (1) or they complex zinc itself forming a neutral complex which facilitates the mucosal uptake and absorption of zinc (7,8). The demonstration of an in vitro impairment of zinc uptake by small intestine mucosal biopsies from patients with AE (6) makes the latter hypothesis more tenable and we have investigated this using liposomes as model membranes.

MATERIALS AND METHODS

Liposomes comprising egg yolk phosphatidyl choline, phosphatidic acid and cholesterol in the molar ratios of 61, 5 and 33 with or without Diodoquin were prepared, as detailed elsewhere (9), by pipetting appropriate aliquots of stock chloroform solution into a glass tube. These were dried under vacuum and then totally resuspended in a solution of NaCl (94 mM) and Tris-chloride (5 mM) buffered at pH 7.5 containing ^{65}Zn labelled $ZnCl_2$ (80 μM). After equilibration under N_2 excess radiotracer was removed by passage of the lipid suspension through a Sephadex G50 medium grade column (Pharmacia). 2ml aliquots of lipid eluate was placed into dialysis bags (Visking 8/32) and these were transferred to stoppered glass tubes containing an identical but non-radiolabelled solution. The tubes were kept at 30°C in a constant temperature water bath and shaken throughout the

experiment. Samples of dialysate were taken at known intervals and their radioisotope content counted. The percentage of initial count remaining trapped within the liposomes was calculated.

The effect of pH on the complexation of zinc by Diodoquin was studied by measuring its extraction from a citrate buffered aqueous zinc chloride solution (50 µmol/l of known pH into an equal volume of a solution of Diodoquin in chloroform (2 mmol/l). After mixing the two phases for two minutes the zinc content of the aqueous phase was determined by atomic absorption spectroscopy (Perkin Elmer 306) and the percentage extracted was calculated by comparison with control systems containing no Diodoquin.

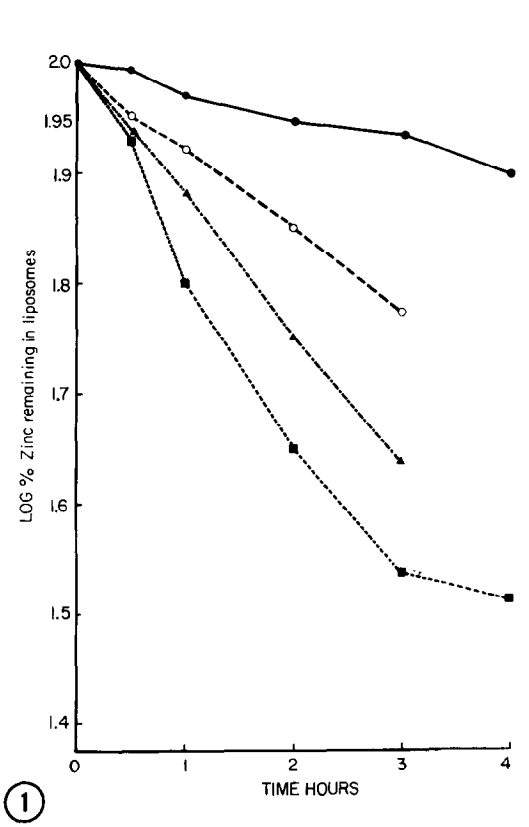
All chemicals were of the purest grade supplied by British Drug Houses. $^{65}ZnCl_2$ was obtained from the Radiochemical Centre, Amersham, UK and the Diodoquin was a gift from May & Baker UK Ltd. The preparation of phosphatidyl choline and phosphatidic acid is described elsewhere (10).

RESULTS AND DISCUSSION

Diodoquin was found to increase the permeability of these pure lipid membranes to ^{65}Zn in a dose dependent manner (Fig.1). The incorporation of 1 µmol 8-hydroxyquinoline per 100 µmols of lipid within the membrane induced a similar permeability to that caused by the same amount of Diodoquin. The relative impermeability to sucrose in the presence of Diodoquin (Fig. 2) excludes a significant non-specific effect on the membrane.

In the partition study Diodoquin was found to extract 90% of zinc from a 50 µM zinc chloride aqueous solution at pH 7.4 into a chloroform phase. The pH for 50% extraction was 6.5.

Our results support the hypothesis that Diodoquin enhances zinc absorption in AE by forming lipid-soluble complexes which enable the cation to traverse lipid bilayers. In this way it behaves as a pH dependent ionophore (11). The similar activity of 8-hydroxyquinoline in this study and the therapeutic efficacy of



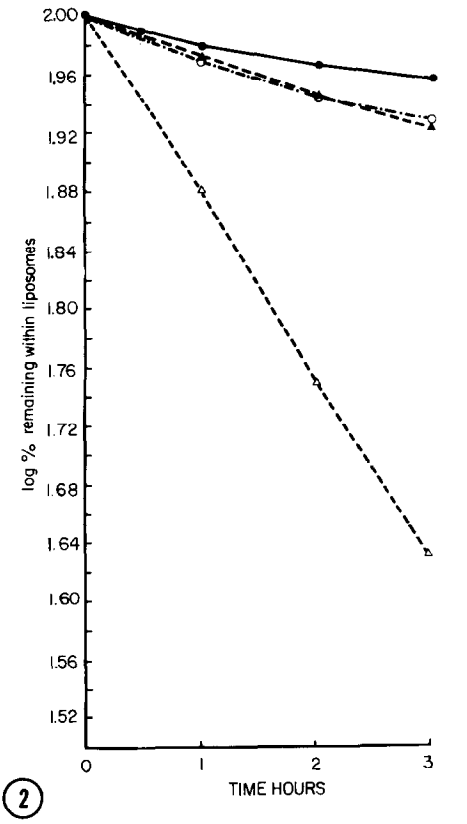


Figure 1 The effect of incorporating 0.5 o, 1.0▲ and 5.0■ µmol of Diodoquin per 100 µmol of total lipid on the permeability of liposomes to $^{65}ZnCl_2$ compared with controls containing no Diodoquin●

Figure 2 The percentage of radiolabel remaining in liposomes containing Diodoquin (1 µmol/100 µmol lipid), suspended in ^{14}C labelled sucrose o (1 mM) and ^{65}Zn labelled $ZnCl_2$Δ(80 µmol) compared with control preparations containing no Diodoquin●▲

its other 5,7-dihalo derivatives in AE suggests that the halogen moiety is unimportant but these do influence aqueous solubility and the pH characteristics of metal complexation (12), both of which may be important in therapeutic use.

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