Eur. J. Clin. Chem. Clin. Biochem.
Vol. 30, 1992, pp. 193–196

Mineral Metabolism in Erythrocytes from Patients with Cystic Fibrosis[1)]

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(Received September 9, 1991/January 10, 1992)

Summary: In patients with cystic fibrosis plasma concentrations of Zn and Mg were unchanged, plasma Ca concentrations were somewhat decreased, but plasma Fe concentrations were drastically reduced; the ratio Cu/Fe in plasma was increased. The Mg and Zn contents of erythrocytes from patients were unchanged. Therefore, the Mg and Zn content of erythrocytes cannot serve for the detection of patients with cystic fibrosis and their heterozygotes, as has been suggested. Cl^--dependent Mg^{2+} efflux from Mg^{2+}-loaded erythrocytes was not affected in cystic fibrosis. Na^+-dependent Mg^{2+} efflux was increased only in erythrocytes from patients with the most severe clinical symptoms.

Introduction

It has recently been reported that erythrocytes from children with cystic fibrosis had significantly lower median concentrations of Na, Mg and Zn, and a higher median concentration of Ca than those of healthy control children and the parents of the cystic fibrosis children (1). Based on these results it was suggested that an analysis of these elements can distinguish cystic fibrosis heterozygotes from healthy controls and that the changed "erythrocyte concentrations of these elements reflect a primary defect of the cystic fibrosis disease and very likely an impairment of Cl^- transport function related to the DF_{508} mutation in the cystic fibrosis gene" (1).

In preceding experiments we have characterized Mg^{2+} efflux from erythrocytes. Mg^{2+} efflux from erythrocytes comprises amiloride-sensitive Na^+/Mg^{2+} antiport and Na^+-independent Mg^{2+} efflux which is combined with Cl^- efflux for charge compensation. The latter can be inhibited by high extracellular Cl^- concentration and by 4-acetamido-4′-isothiocyanatostilbene-2,2′-disulphonic acid (2, 3). As an additional proof of the reduced erythrocyte Mg content, and in view of the fact that a part of Mg^{2+} efflux depends on a functioning Cl^- transport, which might be disturbed in cystic fibrosis, we measured the components of Mg^{2+} efflux from erythrocytes of patients with cystic fibrosis.

Materials and Methods

Patients

Heparinized blood was taken from 15 patients with cystic fibrosis (6 males, 9 females, age range 5–22 years; mean 16). The patients were treated according to their various clinical symptoms. Additionally, they received 3 dragees/day of a multivitamin/mineral preparation (Eunova®). In addition to other components, each dragee contained 7.3 mg Fe^{2+}. Furthermore, the patients received 40 mg Zn orotate and 100 mg vitamin E per day.

For controls, heparinized blood was taken from 4 healthy controls (age 18–38) and 4 patients (age 3–25) not suffering from cystic fibrosis (atopic dermatitis, neuroses).

Blood was centrifuged at 1000 *g* for 10 min. The plasma and buffy coat were aspirated. Plasma was taken for the measurement of Mg, Ca, Zn, Fe and Cu by atomic absorption spectrophotometry (AAS, Philips, SP 9). The erythrocytes were washed twice with 150 mmol/l KCl, then haemolysed by adding 750 µl H_2O. An aliquot (50 µl) of the haemolysate was taken for determination of haemoglobin by the cyanomethaemoglobin

[1)] With financial support by the Deutsche Forschungsgemeinschaft (Sfb 174).

method. The remainder was deproteinized by addition of 50 µl 750 g/l trichloroacetic acid, centrifuged, and the supernatant taken for measurement of erythrocyte Mg and Zn by AAS after dilution with 100 g/l trichloroacetic acid/1.75 g/l $LaCl_3$.

The major part of the erythrocytes was taken for the measurement of Mg^{2+} efflux, as already described (4). The cells were loaded with Mg^{2+} by incubating a cell suspension (cell volume fraction 0.1) for 30 min at 37 °C in KCl medium (in mmol/l: 140 KCl, 12 $MgCl_2$, 50 sucrose, 5 glucose, 30 Hepes/Tris, pH 7.4) with the addition of 6 µmol/l of the cation ionophore A23187 dissolved in dimethyl sulphoxide. For removal of the ionophore, the cells were incubated four times in KCl medium plus 10 g/l bovine serum albumin for 10 min at 37 °C. The KCl medium was removed by washing the cells twice with cold (4 °C) sucrose medium (in mmol/l: 350 sucrose, 5 glucose, 30 Hepes/Tris, pH 7.4).

Mg^{2+} efflux was measured by reincubating a cell suspension (cell volume fraction 0.1) at 37 °C in Mg^{2+}-free NaCl medium (substitution of KCl in KCl medium by 140 mmol/l NaCl), Mg^{2+}-free sucrose medium with or without 30 µmol/l of the anion exchange inhibitor 4-acetamido-4′-isothiocyanatostilbene-2,2′-disulphonic acid (Sigma), or Mg^{2+}-free choline Cl medium (substitution of KCl in KCl medium by 140 mmol/l choline Cl).

At the beginning of reincubation and after 30 min, 0.5 ml aliquots of the cell suspensions were centrifuged for 1 min at 10 000 *g*. Aliquots (100 µl) of supernatants were diluted with 1 ml 100 g/l trichloroacetic acid/1.75 g/l $LaCl_3$, and Mg^{2+} was measured by AAS. Mg^{2+} efflux was calculated from the increase of Mg^{2+} in the reincubation media. Mg^{2+} efflux was expressed in relation to cell volume. Cell concentration was determined by the haematocrit, and by measuring the haemoglobin released by cell lysis.

Results and Discussion

Mineral content of plasma and erythrocytes

Plasma concentrations of Mg and Zn were not significantly different in controls and patients with cystic fibrosis. The Ca concentration in the plasma of cystic fibrosis patients was 0.1 mmol/l lower than in the controls, but it was within the normal range. Some cystic fibrosis patients with expressed maldigestion-malabsorption showed a reduction in plasma Mg. These patients exhibited clinical symptoms of hypomagnesaemia, e. g. paraesthesias.

Plasma Fe concentration was reduced in cystic fibrosis, although the patients had received Fe supplementation for several weeks. Plasma Cu was increased. The increase in Cu was not significant. However, there was a significant difference in the Cu/Fe ratio between controls and cystic fibrosis patients (tab. 1). This effect may represent an acute phase reaction due to infections.

Mg and Zn concentrations in erythrocytes were not significantly different in controls and cystic fibrosis patients (tab. 2); this finding contradicts published data (1).

Tab. 2. Mg and Zn content of erythrocytes from patients with cystic fibrosis and controls. Mean ± SEM. No statistical significance by unpaired *Student*'s t-test.

	Mg mmol/l cells	Zn mmol/l cells
Controls	2.28 ± 0.13	0.16 ± 0.01
Cystic fibrosis	2.43 ± 0.08	0.15 ± 0.01

A possible explanation for this discrepancy may be a better alimentary supply of Mg and Zn in the cystic fibrosis patients in our study, which were supplemented with 6.3 mg Zn/day as Zn orotate. Since the authors of the previous study (1) did not measure serum Mg and serum Zn concentrations and since maldigestion-malabsorption is common in cystic fibrosis, this assumption cannot be ruled out. Probably, the decrease in erythrocyte Mg and Zn is only a secondary complication of cystic fibrosis, as already suggested for Zn in cystic fibrosis (5).

Another possible reason for the difference between our findings and those of the previous study may be the method used to determine Mg and Zn. As measured by particle-induced X-ray emission, Mg and Zn in control erythrocytes amounted to 2.84 and 0.34 mmol/kg *dry weight* (1), whereas the Mg and Zn contents of erythrocytes measured by atomic absorption spectrophotometry were 2.3 mmol/l *cells* for Mg (6, 7, 8, 9) and 0.15 mmol/l *cells* ((10), tab. 2) or 0.2–0.3 mmol/l *cells* for Zn (7, 8). Measurement of erythrocyte Ca was omitted in the present investigation, because the published values for erythrocyte Ca show wide variations.

The true erythrocyte Ca content is extremely low (1 µmol/l cells (6)) and is sensitive to various contaminations (6). The major part of erythrocyte Ca is bound to the external site of the erythrocyte membrane and the extent of its removal varies with the washing procedure (6).

Tab. 1. Concentration of Mg, Ca, Zn, Fe and Cu in plasma of patients with cystic fibrosis and controls. Mean ± SEM. Statistical significance by unpaired *Student*'s t-test. [a], $p < 0.05$, [b], $p < 0.01$

	Mg mmol/l	Ca mmol/l	Zn µmol/l	Fe µmol/l	Cu µmol/l	Cu/Fe
Controls	0.74 ± 0.03	2.17 ± 0.03	11.8 ± 0.7	16.7 ± 2.6	19.4 ± 1.5	1.16 ± 0.20
Cystic fibrosis	0.72 ± 0.02	2.07 ± 0.03[a]	11.6 ± 0.5	9.1 ± 1.2[a]	26.6 ± 3.5	2.92 ± 0.54[b]

Mg^{2+} efflux

As an additional test for a potential decrease in erythrocyte Mg in patients with cystic fibrosis, we measured Mg^{2+} efflux from Mg^{2+}-loaded erythrocytes. There was an insignificant increase in Na^+-dependent Mg^{2+} efflux, which operates via Na^+/Mg^{2+} antiport (tab. 3). However, when the patients were grouped according to their clinical symptoms (1 = mild, 2 = medium, 3 = severe clinical symptoms (according to *Shwachman & Kulczyzki* (11)), erythrocytes with high Na^+/Mg^{2+} antiport were derived from patients with severe clinical symptoms of cystic fibrosis (fig. 1, tab. 4). Probably, there is a structural alteration in the erythrocyte membrane of these patients, but the mechanism is still unclear. Since most of these patients inhaled amiloride, which inhibits Na^+/Mg^{2+} antiport (7), it may be speculated whether the increased Na^+/Mg^{2+} antiport in these patients is a compensatory effect. However, the patient with the highest rate of Na^+/Mg^{2+} antiport did not tolerate amiloride inhalation. Na^+-independent Mg^{2+} efflux, which operates in combination with Cl^- efflux, and which is inhibited by 4-acetamido-4′-isothiocyanatostilbene-2,2′-disulphonic acid and Cl^-, was not changed in cystic fibrosis (tab. 3).

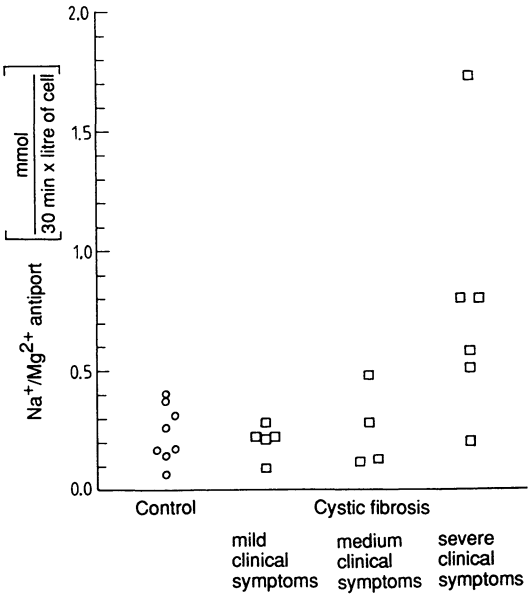

Fig. 1. Na^+/Mg^{2+} antiport in erythrocytes from controls and patients with cystic fibrosis, grouped according to the severity of disease.
Values represent the difference of Mg^{2+} efflux in NaCl medium minus Mg^{2+} efflux in choline chloride medium.

Tab. 3. Mg^{2+} efflux from erythrocytes from patients with cystic fibrosis and controls in NaCl, choline chloride and sucrose medium. 4-Acetamido-4′-isothiocyanatostilbene-2,2′-disulphonic acid (SITS): 30 µmol/l.
Mg^{2+} efflux values in mmol/30 min × l cells. Mean ± SEM. No statistical significance by unpaired *Student*'s t-test.

Medium	NaCl[a]	Choline chloride[a]	Sucrose	Sucrose + SITS
Controls	0.34 ± 0.03	0.10 ± 0.01	0.88 ± 0.05	0.18 ± 0.02
Cystic fibrosis	0.55 ± 0.10	0.11 ± 0.01	0.92 ± 0.05	0.16 ± 0.01

[a] Na^+/Mg^{2+} antiport (Mg^{2+} efflux in NaCl minus Mg^{2+} efflux in choline chloride medium) is 0.24 ± 0.04 mmol/30 min × l cells for control and 0.44 ± 0.12 mmol/30 min × l cells for cystic fibrosis patients.

Tab. 4. Age (years), body weight (kg), sex, severity of disease (score 1 – 3 = mild, medium or severe clinical symptoms); erythrocyte Mg- and Zn-concentration (E-Mg and E-Zn, mmol/l cells); plasma Mg-concentration (P-Mg, mmol/l); plasma Zn-concentration (P-Zn, µmol/l) and Na^+/Mg^{2+} antiport (mmol/30 min × l cells) of the patients with cystic fibrosis in the study.

Age	Body weight	Sex	Score	E-Mg	E-Zn	P-Mg	P-Zn	Na^+/Mg^{2+} antiport
5	18	♂	1	2.76	0.19	0.79	16.7	0.28
10	30	♀	1	3.08	0.14	0.81	11.9	0.09
13	58	♀	1	2.73	0.17	0.74	11.5	0.22
15	62	♂	1	2.34	0.13	0.81	13.2	0.22
20	62	♂	1	2.69	0.15	0.84	9.6	0.21
9	48	♀	2	2.50	0.19	0.60	9.8	0.28
10	29	♀	2	1.92	0.12	0.69	10.3	0.12
10	30	♂	2	2.17	0.13	0.75	11.1	0.13
22	53	♀	2	2.87	0.16	0.80	10.6	0.48
15	36	♀	3	2.30	0.15	0.83	11.5	0.80
16	29	♀	3	2.80	0.16	0.67	10.3	0.80
19	50	♀	3	2.28	0.12	0.66	15.9	0.51
22	50	♂	3	1.96	0.14	0.70	11.2	0.58
23	49	♀	3	2.40	0.18	0.54	10.9	1.73
23	52	♂	3	2.29	0.15	0.61	9.4	0.20

The biochemical defect in cystic fibrosis is a mutation of the cAMP-dependent Cl^- channel in airway and intestinal epithelia (12). Cl^--transport in erythrocytes is performed by an anion exchange protein (band 3, capnophorin) (13), which is different from the Cl^- channel of secretory cells. This explains, why the Na^+-independent (Cl^--dependent) Mg^{2+} efflux is unchanged in erythrocytes from cystic fibrosis patients.

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