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Clinical paper

Improvement of neurobehavioral disorders in children supplemented with magnesium-vitamin B6

I. Attention deficit hyperactivity disorders

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Abstract. Some previous studies have reported the involvement of magnesium (Mg) deficiency in children with ADHD syndrome. In this work, 40 children with clinical symptoms of ADHD were followed clinically and biologically during a magnesium-vitamin B6 (Mg-B6) regimen (6 mg/kg/d Mg, 0.6 mg/kg/d vit-B6) which was set up for at least 8 weeks. Symptoms of ADHD (hyperactivity, hyperemotivity/aggressiveness, lack of attention at school) were scored (0-4) at different times; in parallel, intraerythrocyte Mg^{2+} (Erc-Mg) and blood ionized Ca^{2+} (i-Ca) were measured. Children from the ADHD group showed significantly lower Erc-Mg values than control children (n = 36). In almost all cases of ADHD, Mg-B6 regimen for at least two months significantly modified the clinical symptoms of the disease: namely, hyperactivity and hyperemotivity/aggressiveness were reduced, school attention was improved. In parallel, the Mg-B6 regimen led to a significant increase in Erc-Mg values. When the Mg-B6 treatment was stopped, clinical symptoms of the disease reappeared in few weeks together with a decrease in Erc-Mg values. This study brings additional information about the therapeutic role of a Mg-B6 regimen in children with ADHD symptoms.

Keywords: hyperactive children, attention deficit hyperactivity disorders, magnesium-vitamin B6 diet, intraerythrocyte magnesium, ionized Ca^{2+}

Attention deficit/hyperactivity disorder (ADHD) is the most common neurobehavioral disorder presenting for treatment in youth. Children with ADHD are "a group at risk" as far as their further emotional and social development and educational possibilities are concerned [1]. An effective intervention for many hyperactive children, beside methylphenidate and other psychostimulant drugs, is the use of vitamin B6 (pyridoxine) and magnesium (Mg^{2+}). For over 30 years, parents have given high doses of pyridoxine and Mg^{2+} to their children and have observed decreased physical aggression and improved social responsiveness. However, until now very few studies reported a possible association between magnesium supplementation, ADHD (attention deficit hyperactivity disorders) symptoms, and Mg^{2+} status of the children: the first one from Liebscher *et al.* [2] suggested that patients with ADHD should be consid-

ered as potentially Mg-deficient as regard to a wrong interpretation of the serum Mg test (tetanic patients have lower Mg values than normals). Other studies from Kozielec *et al.* reported for the first time an intra-erythrocyte magnesium deficiency in ADHD children [3, 4]. We recently published similar data [5]. However, it is not a true "Mg deficiency" with clinically associated respiratory repletion, as that observed in familial hypomagnesaemia with secondary hypocalcemia [6]. More precisely, it may be called "intracellular Mg deficiency", affecting mainly neural transmission, very sensitive to such ionic variations.

In order to study the relationships between hyperactivity symptoms and Erc-Mg levels, we designed an open study on 40 children with ADHD syndrome. For ethical reasons, performing a double-blind study against either psychostimulants (methylphenidate) or placebo was excluded: parents would be opposed. In addition, psychostimulants have been found to alter magnesium homeostasis [7]. Our results showed a statistically significant improvement of the symptoms after Mg-B6 supplementation, together with a rise in Erc-Mg values.

Patients and methods

Seventy-six children were followed over a period of at least 6 months: 40 children (mean age: 6.49 years; 13 girls and 27 boys) presented clinical symptoms of ADHD (as described in the DSM-IV diagnostic criteria manual [8], after discussion with parents and teachers, and after psychiatric/neurologic examinations) (ADHD group). The control group contained 36 children (mean age: 4.37 years, 14 girls and 22 boys) somatically and behaviourly healthy; these children did not received any Mg-B6 therapy. They were selected for their normal behavior at school.

Each clinical symptoms of ADHD (the tripod hyperexcitability, hyperemotivity/aggressiveness, lack of school attention) was scored between 0 to 4: for hyperactivity, score 0: absence of symptom; score 4: child hyperactive; for attention in school, score 0: child very attentive at school, score 4: child poorly attentive; for hyperemotivity/aggressiveness, score 0: absence of aggressiveness, score 4: child very aggressive and hyperemotive. A Mg-B6 regimen (6 mg/kg/d for magnesium and 0.6 mg/kg/d for vit. B6) was established for at least six months in all ADHD children. No other medical treatment was given before and during the Mg-B6 period.

Serum Mg^{2+} (S-Mg) and intra-erythrocyte Mg^{2+} were measured by a colorimetric assay (chlorophosphonazo III) [9] (Erc-Mg) in an INTEGRA automate (Roche Diagnostics) and blood ionized Ca^{2+} concentrations by electrometric assay (i-Ca) (Bayer Diagnostics). To perform Erc-Mg measurements, red blood cells (RBC) were washed 3 times in 0.9% NaCl, centrifuged, and RBC (1 mL pellet) were lysed in 2 mL water for 15 min at + 4 °C. Then, 1 mL 20% trichloracetic acid was added, the mix was stirred on vortex and centrifuged. The supernatant (1/4 dilution) was used to measure Erc-Mg. When repeated four times in healthy children at one month periods, Erc-Mg values varied by 12% around the initial value. This method was adapted on an INTEGRA automate after calibration with the atomic absorption assay. Biological parameters, including s-Mg, Erc-Mg, and i-Ca, were measured at the first clinical visit of the child; then again, after two months treatment. The following evaluations depended on the frequency of the visits (every six months, for instance). Of course, the control group, only containing healthy children, was not treated with Mg-B6.

Statistics

All statistical analyses were done after testing all variables of interest to determine whether they were approximately normally distributed: two different types of tests for normality were used; Shapiro-Wilk and Shapiro-Francia. Since the majority of the variables are not normally distributed, the non-parametric paired Wilcoxon signed-rank test was used to compare values between before and after treatment. To compare Erc-Mg values between ADHD and control children the non-parametric Mann & Whitney test was used. Significance at $p < 0.05$.

Results

Table 1 reports mean values for biological data of ADHD children before and after Mg-B6 supplementation. All statistical comparisons are reported in *table 2*.

Erc-Mg values are lower in the ADHD group as compared to the control group

While s-Mg did not statistically differ between ADHD and control groups (data not shown), Erc-Mg values were lower in ADHD as compared to those found in control children (2.05 ± 0.3 mmol/L, $n = 41$ *versus* 2.73 ± 0.23 mmol/L, $n = 36$; $p < 0.01$) (*figure 1* and *table 2*). In contrast, i-Ca decreased slightly but not significantly (1.22 ± 0.06 mmol/L *versus* 1.25 ± 0.05 mmol/L for controls; $p = 0.2966$) *(table 2*A*)*. However, a statistically significant posi-

Table 1. Biological parameters of the study. Mean, SD, median, and range of each parameter (Erc-Mg and i-Ca in mmol/L) measured in control and in ADHD children before and after Mg-B6 treatment are reported.

		Number of values	Mean (mmol/L)	SD	Median (mmol/L)	Range
Erc-Mg	Controls	36	2.76	0.26	2.71	1.01
	Before	40	2.05	0.29	2.09	1.23
	After	17	2.35	0.37	2.31	1.46
Ca^{2+}	Controls	36	1.25	0.05	1.24	0.22
	Before	38	1.22	0.06	1.21	0.25
	After	18	1.21	0.11	1.22	0.25

tive correlation was found between Erc-Mg and i-Ca values in both controls and ADHD children ($p = 0.032$).

Erc-Mg values increased under Mg-B6 supplementation

When patients received a Mg-B6 supplementation for at least two months, a significant rise in Erc-Mg values was observed in ADHD (2.32 ± 0.41 mmol/L *versus* 2.05 ± 0.3 mmol/L, $p = 0.004$), but these values were still lower than for controls *(figure 1)*. *figure 2* reports changes in Erc-Mg values during Mg-B6 therapy in three cases of ADHD: the rate of increase in Erc-Mg values was about 2 months. When Mg-B6 supply was stopped, Erc-Mg values returned to low levels in about 2 months. Administration or suppression of oral Mg-B6 therapy caused respectively a rise and a decrease in Erc-Mg values. In addition, in patients where i-Ca levels were lower than for controls ($n = 10$), the Mg-B6 supplementation induced a significant rise in both i-Ca and Erc-Mg values ($p = 0.0113$ and $p = 0.0107$, respectively) *(table 2*B*)*.

Evolution of clinical symptoms under supplementation

In almost all cases of ADHD, the Mg-B6 treatment for at least 2 months significantly modified the clinical symptoms of the disease: namely, hyperactivity and hyperemotivity/aggressiveness were reduced and school attention was improved *(figure 3)*. A statistical analysis of the data (chi-2 test) showed that populations of scored values were significantly different ($p < 0.0001$) before and after Mg-B6 treatment (chi-2 values for hyperactivity, school attention, and hyperemotivity/ aggressiveness: 47.1, 17.2, 17.9, respectively; Wilcoxon signed rank test also evi-

Table 2. Statistical comparison Erc-Mg and i-Ca values in the different groups. Erc-Mg values were obtained after lysis of red blood cells and colorimetric assay as described in *Patients and Methods* section. I-Ca values came from specific electrode measurements. In (**A**), statistical comparison was done using unpaired Mann & Whitney non-parametric test. In (**B**), comparison was done by a paired Student's t-test after one factor variance analysis. Statistical significance at $p < 0.05$

A) Statistical analysis of Erc-Mg and i-Ca in controls children and in ADHD children before treatment.

B) Influence of Mg-B6 therapy on Erc-Mg and i-Ca values in children who exhibited i-Ca values lower than controls.

A	**Controls**	**Before treatment**	**Mann & Whitney test**
i-Ca^{2+}	1.25 ± 0.05 (n = 36)	1.22 ± 0.06 (n = 38)	U = 528, P > U = 0.667, NS
Erc-Mg	2.76 ± 0.26 (n = 36)	2.05 ± 0.29 (n = 40)	U = 9, P > U = 0.008
B	**Before treatment (n = 11)**	**After treatment (n = 11)**	**Variance analysis + paired t-test**
i- Ca^{2+}	1.16 ± 0.05	1.24 ± 0.07	F = 6.97 p = 0.0157 t = 3.09 p = 0.0113
Erc-Mg	1.90 ± 0.36	2.33 ± 0.43	F = 6.46 p = 0.0194 t = 3.12 p = 0.0107

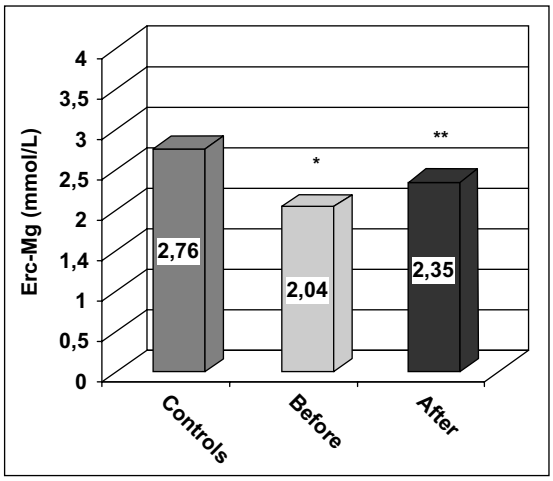

Figure 1. Erc-Mg and i-Ca (mmol/L) mean and SEM values in the two groups of children: normals (n = 36), ADHD (n = 40). ERC-Mg values (mmol/L) were obtained after lysis of red blood cells and colorimetric assay as described in *Patients and Methods* section. I-Ca values came from specific electrode measurements. Comparison of values was done by the paired non-parametric Wilcoxon signed rank test and the non-parametric Mann & Whitney test. Statistical significance at $p < 0.05$. * Comparison Erc-Mg controls/Erc-Mg before: $p = 0.004$. ** Comparison Erc-Mg before/Erc-Mg after: $p = 0.008$. Comparison Erc-Mg controls/Erc-Mg after: NS.

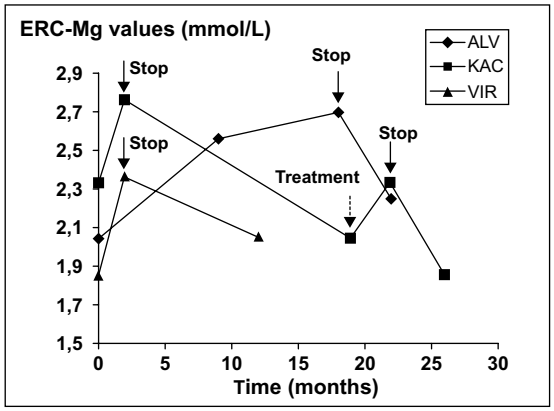

Figure 2. Erc-Mg variations during Mg-B6 therapy in three different ADHD children: ERC-Mg values were followed over a period of 27 months during Mg-B6 therapy. In ALV, the treatment was installed for 18 months, then stopped. In VIR, after 2 months, the treatment was stopped. In KAC, the treatment was for two months, then stopped for 15 months; it was started again (hatched arrow) for 3 months, then stopped.

denced a significant difference for the three groups). When the magnesium treatment was stopped, clinical symptoms of the disease reappeared in few weeks.

Erc-Mg values/clinical symptoms relationships

A majority of children improved under treatment together with a rise in Erc-Mg values (11/19), while only 8/19 did not improve or improved with a decrease in Erc-Mg values. Unfortunately, when we tried to correlate changes in Erc-Mg values (ratio Erc-Mg after treatment *versus* Erc-Mg before treatment) to changes in clinical symptoms (difference of scored values between after treatment and before treatment), no statistically significant correlation appeared. However, the improvement of hyperactivity appeared to be positively associated to high Erc-Mg values measured before treatment (*figure 4*). So, the more Erc-Mg values are elevated before treatment (although lower than controls), the more hyperactivity was improved ($p = 0.08$). This observation could be interpreted by the fact that, when tissues are depleted in Mg, the time (or the dosage) required to restore normal values became more important. Since measurements were done at the same time, these parameters (duration and dosage) have not been taken into account in this study.

Discussion

Magnesium is essential for a number of physiological and biochemical central and peripheral processes. In the brain, traumatic injury causes a decline in magnesium concentrations, focally as well as in blood circulation, and contributes to the development of neurologic deficit [10]. Brain ischemia causes a decline in intracellular free Mg concentrations and magnesium salt administration could improve motor outcome [11]. One of the most important modes of action of magnesium is to inhibit the glutamate N-methyl-aspartate channel, associated to an influx of calcium and, in turn, an excitotoxic cell death and apoptosis [10]. So, while Mg^{2+} has been shown to be a non-specific inhibitor of calcium channels, it could act as NMDA channel inhibitor [12]. In the same way, Mg^{2+} could influence catecholamine signaling in the brain [13].

In our study, a slight but significant intra-erythrocyte Mg^{2+} depletion was evidenced in ADHD patients together with a concomittant decrease in i-Ca concentrations. As we know, Mg^{2+} is essential

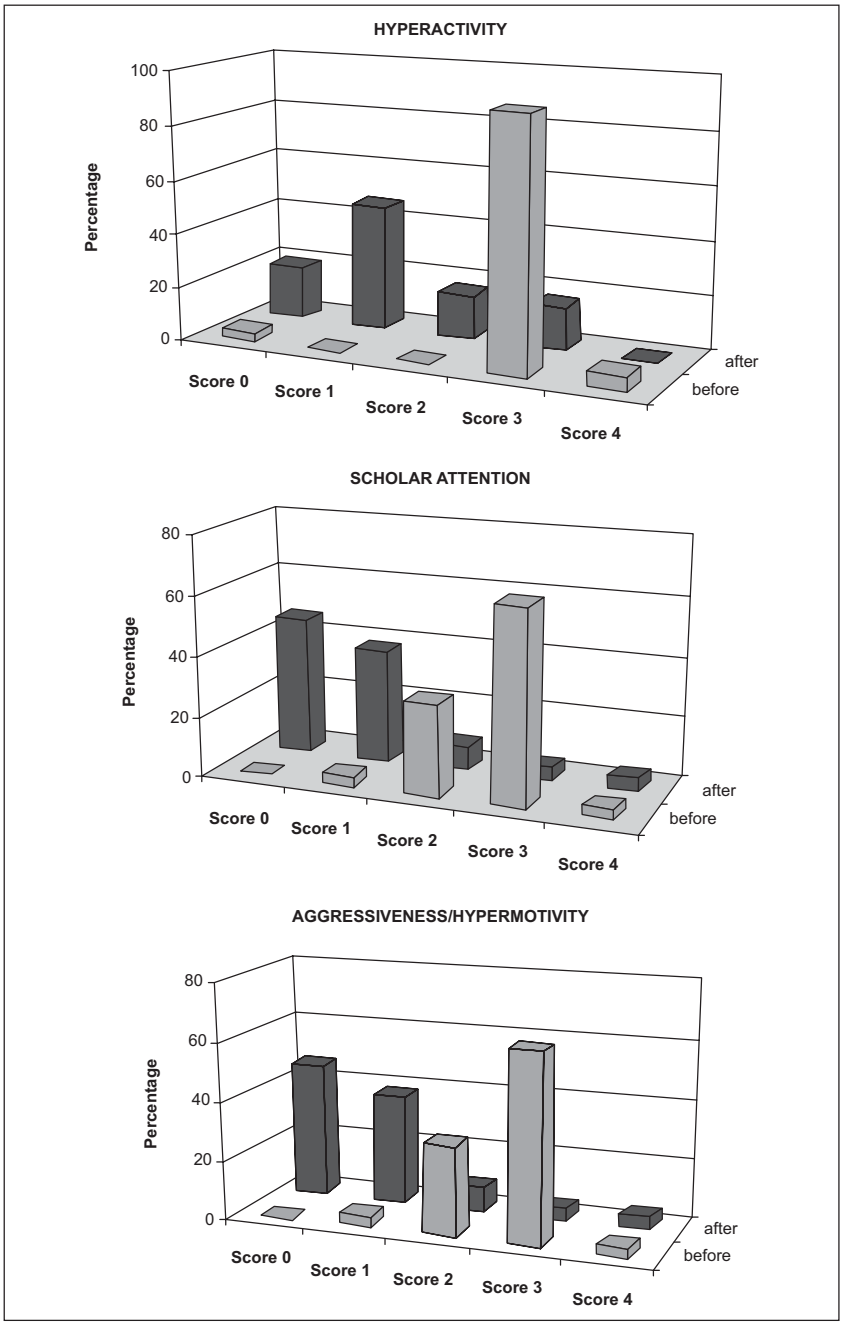

Figure 3. Influence of Mg-B6 therapy on clinical symptoms of ADHD children. Hyperactivity, school attention and hyperemotivity/aggressiveness were scored from 0-4 in children before and after at least 2 months Mg-B6 therapy (for hyperactivity, score 0: absence of symptom; score 4: hyperactive child; for school attention, score 0: child attentive at school, score 4: poorly attentive child; for hyperemotivity/aggressiveness, score 0: absence of aggressiveness, score 4: very aggressive and hyperemotive child). Comparison of scored values was done by the paired non-parametric Wilcoxon signed rank test. Statistical significance at $p < 0.05$.

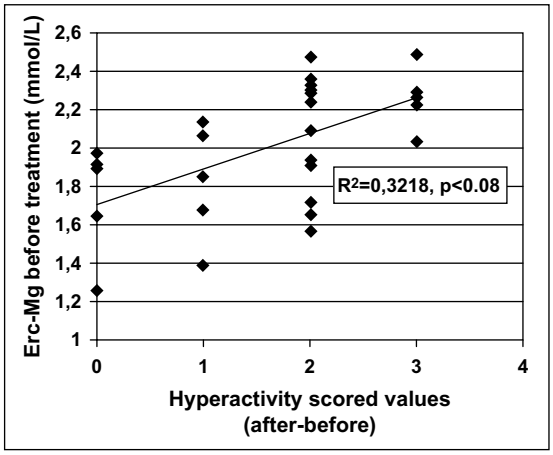

Figure 4. Correlation between Erc-Mg before Mg-B6 treatment and improvement of hyperactivity symptoms. Hyperactivity was scored between 0-4 in ADHD children before and after Mg-B6 therapy installed for at least 2 months. Values reported are the difference in scored values (after–before treatment). Erc-Mg was measured before treatment as described in *figure 1* (expressed in mmol/L).

for normal central activity and Erc-Mg could be representative of intracellular Mg concentrations: a decrease in Erc-Mg without changes in s-Mg concentrations could be interpreted as an alteration of a magnesium transporter (Na^+/Mg^{2+} exchanger) in erythrocytes with concomitant incidence on neuronal Mg concentrations. The impairment to achieving normal Erc-Mg values under Mg-B6 treatment supports this hypothesis. In addition, Mg pidolate supplementation was found to decrease Na^+/Mg^{2+} exchanger activity with a concomitant rise in Mg^{2+} and K^+ content of erythrocytes in sickle cell disease [14].

Erc-Mg has been described as a controversial biological parameter for the monitoring of Mg^{2+} deficiency: in contrast to Borella *et al.* [15] who consider Erc-Mg as a suitable index, Basso *et al.* [16] present Erc-Mg as not useful in the monitoring of individual changes: we think that, in this last study, a 3-week treatment with Mg^{2+} without B6 was too short to induce a durable increase in Erc-Mg (vitamin B6 was described to enhance Mg^{2+} entry through the cell). Anyway, in our study, Erc-Mg measurements were standardized and they appear as a potent indicator of cellular magnesium deficiency.

Ca^{2+} and Mg^{2+} cellular contents classically followed the same pathway: when Mg^{2+} increased, Ca^{2+} also increased. This can explain the significant correlation between Erc-Mg and i-Ca values as well as the fact that in children who have low i-Ca values, Mg therapy increased i-Ca levels. It can be hypothesized that a genetic factor, which modulates Na^+/Mg^{2+} exchanger activity, may be important in the regulation of Mg metabolism [17].

We also found that increased hyperactivity and decreased school attention were associated to decreased Erc-Mg values: this observation was supported by the fact that Mg-B6 supplementation induced a rise in Erc-Mg values and a concomitant improvement of the clinical symptoms. What are the respective roles of pyridoxine and Mg^{2+} in these observations? Mg^{2+} is classically associated to pyridoxine to decrease the irritable side-effects of the B6 therapy. We show here evidence of the role of Mg^{2+} itself in this therapy. Previous data support this observation: in ADHD disorders, in which disruptive behaviour with hyperactivity was found, psychostimulants are used to improve mental health, probably through increasing synaptic noradrenaline activity. In children who received methylphenidate, Schmidt *et al* [7] found a significant increase (6%) in plasma Mg^{2+} concentrations depending on the dosage of the drug, showing a relationship between improvement of hyperactivity and Mg^{2+} metabolism. More recently, in autistic children with behavioural disorders and hyperactivity, Zilbovicius *et al.* [18, 19] using positive emission tomography (PET) have shown, in 76% of the children examined, a significant decrease in cerebral blood flow localized at the temporal lobe level. Taken together with the fact that intra-erythrocyte free Mg^{2+} is associated to increased blood pressure [20] and that brains from rats fed with a low Mg^{2+} diet are more susceptible to permanent brain focal ischemia [21], we can hypothesize that intracellular Mg^{2+} deficiency could be responsible, at least in part, for some central activity disorders observed in these children.

The duration of the treatment to get significant improvements seems to be about 8 weeks; since the cause of this deficiency is yet unknown, and since the symptoms reappeared when the Mg-B6 diet was stopped, the treatment must be maintained for a long time. In addition, while it was difficult to find an evident biological link between central disorders and Erc-Mg values, this biological parameter could be used to select, among the large population of children with hyperactive symptoms, a small population with behavioural abnormalities which is relevant to a Mg-B6 diet. It is evident that another accessible Mg^{2+} store, more significant for central disorders, has to be found.

Conclusion

This study brings additional information about the therapeutic role of a Mg-B6 regimen in children with ADHD. This effect seems to be associated, at least in part, to a cellular Mg^{2+} deficiency, as evidenced by intraerythrocyte Mg^{2+} measurements. Installing a Mg-B6 supplementation for some weeks restored higher intraerythrocyte Mg^{2+} values and significantly reduced the clinical symptoms of these diseases. As chronic magnesium deficiency was shown to be associated to hyperactivity, irritability, sleep disturbances, and poor attention at school, magnesium supplementation as well as other traditional therapeutic treatments, could be required in children with ADHD.

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