Magnesium, hyperactivity and autism in children

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Abstract

For many years, magnesium has been described as a crucial factor for cellular activity. In this chapter, a brief overview of pharmacology and genetics of magnesium transport will be followed by a review of clinical and biological studies of Mg^{2+}/VitB6 supplementation in attention deficit/hyperactivity disorder (ADHD) and autism (autistic spectrum disorders family, ASD) in children. Although to date, no study carried out on a rational basis has been published, some experimental and/or clinical works support the hypothesis of a positive effect of such therapy in these pathologies. All the individual observations report a decrease in hyperactivity and a stabilization of scholarly behaviour with treatment. These data strongly support the need for a controlled study to confirm or invalidate these assumptions.

Introduction

Magnesium is the second most abundant intracellular cation in the body; its main action is to regulate enzyme activity, to control the activity of various calcium and potassium channels, and to promote membrane stabilization. It is also responsible for the maintenance of the transmembrane gradients of sodium and potassium. Magnesium depletion is known to be associated with many clinical diseases including hypocalcemia, hypokalemia, cardiac arrhythmias, neuromuscular excitability, hypertension, atherosclerosis, and osteoporosis. Some evidence indicates that magnesium could also be involved in neurological diseases such as attention deficit/hyperactivity disorder and autism. However, no direct study has been published to confirm this assumption.

Attention deficit/hyperactivity disorder (ADHD) and autism (autistic spectrum disorders, ASD; pervasive developmental disorders, PDD) are different neurological disorders which have been described for many years, and in which the involvement of a deficient magnesium pathway could be suspected given the presence of active transport for such cations through transient receptor potential melastatin (TRPM) channels in brain.

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 (Spencer *et al*, 2002). An effective intervention for many hyperactive children, beside methylphenidate and other psycho-stimulant 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, up until today, very few studies have reported a possible association between magnesium supplementation, ADHD (attention deficit hyperactivity disorders) symptoms, and Mg^{2+} status of the children. The first such study from Liebscher et al (Liebscher and Liebscher, 2003) suggested that patients with ADHD should be considered as potentially Mg-deficient as opposed 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 (Kozielec and Starobrat-Hermelin, 1997; Mousain-Bosc *et al,* 2004). We also published similar data (Mousain-Bosc *et al,* 2004, 2006). However, it is not a true "Mg deficiency" with clinically associated respiratory repletion, as that observed in the familial hypomagnesaemia with secondary hypocalcemia (Shalev *et al,* 1998; Mousain-Bosc *et al,* 2004). More precisely, it may be called "intracellular Mg deficiency", affecting mainly

neural transmission, which is very sensitive to such ionic variations.

Another group of neuronal diseases in which magnesium has been implicated is ASD/PDD/ autism. Studies from 18 different research groups have shown that B6 and Mg^{2+} are beneficial to about half of autistic individuals, with no significant adverse effects. Eleven of these studies involved a double-blind placebo design and have documented decreases in behavioral problems, improvements in appropriate behavior, and normalization of brain wave activity and urine biochemistry. There is also evidence that B6 and Mg^{2+} may reduce seizure activity. Parent reports confirm improvement in attention, learning, speech/language, and visual contact. More recently, in a pilot study of a moderate dose multivitamin/mineral supplement for children with autistic spectrum disorder, Adams and Holloway (2004) found significant improvements in sleep and gastrointestinal problems compared to the placebo group. Despite all these data, the intervention of Mg-B6 remains controversial and contradictory studies have been published (Vink, 2001; Helpern, 1993; Mousain-Bosc *et al,* 2006; Shalev *et al,* 1998).

So, new data concerning a possible association between Mg-B6 supplementation, neurobehavioral symptoms, and the Mg^{2+} status of children have been published, opening a new way of research in this domain. This chapter will present a review of essential works published in this domain.

Magnesium transport and pharmacology of magnesium

Magnesium transport

The protein involved in Mg permeable channels belongs to the family of TRPM proteins (Schmidt and Taylor 1988; Chubanov *et al,* 2004). The transient receptor potential (TRP) superfamily consists of a large number of cation channels that are mostly permeable to both monovalent and divalent cations. The 28 mammalian TRP channels can be subdivided into six main subfamilies: the TRPC (canonical), TRPV (vanilloid), TRPM (melastatin), TRPP (polycystin), TRPML (mucolipin) and the TRPA (ankyrin) groups. TRP channels are expressed in almost every tissue and cell type and play an important role in the regulation of various cell functions. Currently, significant scientific effort is being devoted to understanding the physiology of TRP channels and their relationship to human diseases. At this point, only a few channelopathies in which defects in TRP genes are the direct cause of cellular dysfunction have been identified. In addition, mapping of TRP genes to susceptible chromosome regions (e.g., translocations, breakpoint intervals, increased frequency of polymorphisms) has been considered suggestive of the involvement of these channels in hereditary diseases. Moreover, strong indications of the involvement of TRP channels in several diseases come from correlations between levels of channel expression and disease symptoms. Finally, TRP channels are involved in some systemic diseases due to their role as targets for irritants, inflammation products, and xenobiotic toxins. The analysis of transgenic models allows further extrapolations of TRP channel deficiency to human physiology and disease.

Two receptors, TRPM6 and TRPM7, have been identified as Mg^{2+} permeable ion channels and their specificity is due to their link with protein kinase. This is the reason why they are defined as chanzymes. Theses chanzymes are involved in Mg^{2+} reabsorption in kidney and intestine. The intake of Mg^{2+} is driven by a transmembrane potential that facilitates the entry of the cation through the TRPM6 channel at the apical part of epithelial cells. The hypothesis is in favor of a Na-dependent flux through ATP-dependent mechanisms (De Franceschi *et al*, 2000; Nilius *et al,* 2007).

In the kidney, different transport pathways for magnesium exist along the nephron. The majority of filtered magnesium is reabsorbed in the thick ascending limb of Henle`s loop via the paracellular route. Observations using genetic studies in affected individuals disclosed the first molecular components of epithelial magnesium transport: the tight junction protein Paracellin-1 (Claudin-16) which was discovered as a key player in paracellular magnesium and calcium reabsorption in the thick ascending limb of Henle`s loop in the distal convoluted tubule (De Franceschi *et al*, 2000; Nilius *et al,* 2007). Mutations of this protein lead to familial hypomagnesemia with hypercalciuria and nephrocalcinosis, a combined urinary magnesium and calcium wasting which almost invariably leads to progression to end stage renal disease.

The discovery of TRPM receptors involved in Mg^{2+} homeostasis (Borella *et al*, 1993; Montell, 2003) serves to identify by genetic engineering and molecular cloning the genes involved in primary inherited hypomagnesaemia. Several genes encoding proteins are directly involved in renal magnesium handling. The family of protein paracellin 1 (PCLN-1) is directly involved in magnesium and calcium reabsorption in the distal convulated tubule (DCT). So diseases such as hypomagnesaemia with secondary hypocalcaemia (HSH) are observed. Reabsorption of magnesium in the DCT is active and transcellular. The renal magnesium leak in HSH patients is due to the role of TRPM6 for active transcellular magnesium reabsorption in the DCT. So the strong interaction between TRPM6 and TRPM7 involves the apical magnesium channel responsible for its uptake from urine into DCT cells. In HSH patients, renal magnesium wasting not only contributes to the development of hypomagnesemia in the postnatal period, but also prevents an adequate conservation of the absorbed magnesium under supplementation.

Hypocalcemia is due to an inhibition of parathyroid hormone caused by a profound hypomagnesemia. Affected children typically manifest during the first months of life with generalized convulsions or signs of increased neuromuscular excitability like muscle spasms or tetany. Reduced clinical symptoms and the normalization of calcium homeostasis are guaranteed by an immediate intravenous administration of magnesium followed by a long substitution with high oral doses of magnesium.

HSH can be also characterized as an autosomal recessive disease due to the gene mapped on chromosome 9q22. Mutational studies on TRPM 6 receptor have shown the same type of HSH; so TRPM 6 is identified as a Mg^{2+} permeable ion channel that causes a combined defect of intestinal and renal magnesium transport.

TRPM6 protein homologous at 52% to TRPM7 was studied together in order to study their regulation. They were functionally characterized as a constitutively active ion channel permeable to a variety of cations like calcium and magnesium. So, TRPM6 and TRPM7 are closely related members of the TRPM transient receptor potential ion channel family named after its founding member melastatin. The pathophysiologic consequences of a TRPM6 defect for magnesium transport in intestine and distal convoluted tubule are important. In the intestine, an active transcellular transport and a passive paracellular pathway were identified. Theses proteins share the unique feature of an atypical kinase domain at their C-terminus for which they have been termed chanzymes (Basso *et al*, 2000; Schlingmann and Gudermann, 2005). The uptake on apical entry into the epithelial cell is a basolateral extrusion mechanism that couples magnesium export to sodium influx. These transport processes yield a curvilinear kinetic for intestinal magnesium absorption. In addition, it has been described that an increased intake of oral magnesium enhances passive paracellular absorption. So, it can be supposed that HSH patients with defective transcellular magnesium transport are able to achieve relief of symptoms and at least subnormal serum magnesium levels.

The electrophysiological and biochemical analyses identified TRPM7 as an important player in cellular magnesium homeostasis; the critical role of TRPM6 for epithelial magnesium transport emerged from the discovery of loss-of-function mutations in patients with a severe form of hereditary hypomagnesemia, called primary hypomagnesemia with secondary hypocalcemia or HSH. TRPM6 plays an important role as an influx pathway for Mg^{2+} (Schmidt and Taylor, 1988; Chubanov *et al*, 2004).

So, TRP channels have a multifunctional role and are involved in many fundamental cell functions investigated in physiopathology and diseases. The TRP superfamily was related to channelopathies, and involves an activation of membrane cation channel. The TRPM subfamilies are distinct families, whereas both TRPM6 and TRPM7 (serine /threonine kinase) exhibit high/variable permeability to Ca^{2+} and Mg^{2+} and are regulated through intracellular levels of Mg^{2+} and Mg-ATP. The defects in these ions channels is supposed to cause various diseases describes as channelopathies. The genetic defect in TRP channels is identified as the direct cause of hereditary disease. Mutations on the TRPM6 gene are liked to human proteinuric kidney disease. TRPM7, the closest relative of TRPM6, is implicated in some neurodegenerative diseases such as amyotrophic lateral sclerosis and Parkinson's disease.

Involvement of magnesium in membrane stability and gene expression

Membrane stability is influenced by many parameters including ionic conductance, ionic fluxes and magnesium (Ebel and Gunther, 2005; Wolf *et al*, 2009). The effect of Mg^{2+}, due to the activity of the superfamily of TRPs channels, is observed on many membranes. It has been reported that the concentration of extracellular Mg^{2+} can affect blood flow, blood pressure and vascular reactivity in intact mammals (Schmidt and Taylor, 1988, Van der Wiijst et al, 2009). The red cell membrane properties have also been explored: Mg^{2+} regulates stability and extends the gross elasticity of the red cell membrane. Magnesium-depleted cells also undergo structural changes on heating below the temperature at which vesiculation sets in.

There is drastic change in ionic flux through the outer and inner cell membranes both in the impaired membranes of cancer, and in Mg deficiency. Researchers from the School of Public Health at the University of Minnesota have just concluded that diets rich in magnesium reduced the occurrence of colon cancer (Chakraborti *et al,* 2002; Aaron, 2006). In another study (Macdonald *et al,* 2004; Susanna *et al,* 2005) women with the highest magnesium intake evidenced a 40% lower risk of developing cancer than those with the lowest intake of the mineral.

Magnesium linked to the phospholipids and more particularly to phosphatidylserine reduces membrane fluidity, and increases the stability of the membrane of pre-synaptic vesicles. Lecithines (phosphatidylcholines) and phosphatidylserines are brain nutrients, which modulate acetylcholine metabolism, and act stimulating memory. A 2003 study (Demougeot *et al,* 2004) describes the lecithins as promoting the synthesis and the working of neurotransmitters involved in memory processes. The mechanism of beneficial action would be linked to activation by choline of phospholipase systems in the hippocampus.

In obstetric and embryonic research, different magnesium salts were studied, such as $MgCl_2$, Mg-acetate and Mg-citrate; they are known to increase and decrease the membrane stability on the two faces of the amnion. $MgSO_4$, Mg-lactate and Mg-nitrate increased the stability on the maternal side, but decreased it on the fetal side. So, addition of magnesium salts modified the human amniotic membrane stability.

In response to a magnesium deficiency, five genes could be identified by using DNA arrays: osteopontin, the cholecystokinin A receptor, connexin 45, a growth hormone receptor and BAG1. Other fundamental studies characterized in bacteria an 'M-box'. It is a genetic switch that is sensitive to Mg^{2+} cellular levels through the conformation of its newly synthesized RNA. This Mg^{2+} sensing riboswitch controls transcription termination in front of a Mg^{2+} transporter gene. So magnesium acts directly as a genetic regulator in genetic expression of RNA in order to control metal ion homeostasis (De Rose, 2007). As magnesium has been shown to activate gene expression, the mitochondrial genome must be affected. A mild mitochondrial dysfunction, and the treatment, may include administration of the mitochondrial cofactors like magnesium (Martin *et al,* 2007).

Action of magnesium on synaptic facilitation

In 2006, a review article (Billiard, 2006; Demougeot *et al*, 2010*,* 2004) indicated that magnesium is involved in age-related deficits in transmitter release, neuronal excitability, and some forms of synaptic plasticity such as long-term depression of synaptic transmission. Further studies presented by Slutsky et al (2010) show that magnesium is essential for maintaining normal body and brain functions.

Learning and memory are fundamental brain functions affected by dietary and environmental factors. Increasing brain magnesium using a newly developed magnesium compound (magnesium-L-threonate) leads to the enhancement of learning abilities, working memory, and short and long-term memory in rats. Functionally, magnesium increased the number of functional pre-synaptic release sites, while it reduced their release probability. These findings suggest that an increase in brain magnesium enhances both short-term synaptic facilitation and long-term potentiation and improves learning and memory functions. Magnesium impacts upon the release of neurotransmitters, and other mediators or modulators (Slutsky *et al,* 2010).

Magnesium in blood

The study of the regulation of magnesium has gained particular interest in the last decades

thanks to the molecular characterization of specific magnesium transporters and the exploitation of molecular biology techniques to clarify their cellular and physiological function(s). Magnesium could be detected both in the plasma in the cationic form (Mg^{2+}) and in blood cells, namely in erythrocytes. All these assays are today well described and can be realized in many laboratories.

In contrast, experimental tools to trace cellular magnesium and to define its homeostasis in living cells have not witnessed a corresponding progress. It was not until recently that efforts were paid to design more appropriate fluorescent indicators that could translate the advances of live imaging techniques into the field of magnesium research (Trapani *et al,* 2010).

Phosphorus magnetic resonance spectroscopy also offers an opportunity to measure *in vivo* the free cytosolic magnesium [Mg^{2+}] of different tissues. In particular, this technique has been employed in human brain and in skeletal muscle providing new hints on Mg^{2+} homeostasis and on its involvement in cellular bioenergetics. In skeletal muscle it has been shown that the changes in free Mg^{2+} concentration occurring during contraction and in post-exercise recovery are mainly due to the influence of cytosolic pH. The possibility of assessing the free cytosolic [Mg^{2+}] in the human brain offered the chance of studying the involvement of Mg^{2+} in different neurological pathologies, and particularly in those where defective mitochondrial energy production represents the primary causative factor in the pathogenesis. Moreover, it has also been shown that the measurement of brain Mg^{2+} can help in the differential diagnosis of neurodegenerative diseases sharing common clinical features, such as Multiple System Atrophy and Parkinson's disease (Ebel *et al,* 2004; Iotti and Malucelli, 2008).

The magnesium concentrations in plasma and cells could be affected by diet, disease and genetic factors. It is known that carcinogenesis induces magnesium distribution disturbances, which cause magnesium mobilization through blood cells and magnesium depletion in non-neoplastic tissues. Magnesium deficiency seems to be carcinogenic, and in the case of solid tumors, a high level of supplemental magnesium inhibits carcinogenesis. Both carcinogenesis and magnesium deficiency increase the plasma membrane permeability and fluidity. Scientists have in fact found out that there is much less Mg^{2+} binding to membrane phospholipids of cancer cells, than to normal cell membranes (Wolf *et al*, 2008).

A progressive elevation in external Mg^{2+} levels in blood will produce a progressive inhibition of most contractile substances. The contractile response observed upon withdrawal of external Mg^{2+} are dependent upon Ca^{2+} concentrations and polarity of the membrane. This response is not related to an inhibition of Na^{+}, K^{+} ATPase. So, it is known that Mg^{2+}, combined with Na^{+} and K^{+}, play a major role in regulating blood pressure and arteriolar tone (Altura *et al*, 1978).

Moreover, magnesium concentrations in plasma and cells could be affected by genetic disease. The genetic factors involved in the regulation of magnesium homeostasis were studied in low (MgL) and high (MgH) magnesium status strains of mice. In this model, magnesium-deficiency affects both plasma, erythrocyte and urine magnesium concentrations in similar proportions in the two strains (Schlingmann *et al,* 2002; Montell, 2005; Günter, 2007; Ozgo *et al*, 2007)

Clinical symptoms of ADHD syndrome

Clinical cases

ADHD has been described since the beginning of the twentieth century and was called hyperkinesia or “psycho-motor instability”. It would represent 3 to 9 % of the population of children and adults (Feillet-Coudray, 2005; Biederman, 2006). The clinical picture is that of a hyperirritable, impulsive, aggressive child with an attention deficit; the diagnosis was basically done at the sixth year, when the child presented at primary school. These symptoms impair the emotional development and can lead to serious social and family disorders. In adults, statistics show a powerful link between ADHD and serious problems of life. These findings support the idea that, when diagnosed in the community, ADHD is a clinically significant and highly disabling disorder in adults and must be treated very early in children. However, parents’ and children’s histories should be studied. In parents: behavioral disorders, such as hyperactivity, aggressiveness, emotional lability, stress, or maternal spasmophilia. During the pregnancy, several

symptoms can be observed: weariness, stress, anxiety and breakdown, arterial hypertension, muscle cramps, contractions, sleeping disorders, diet deficiency, and twin pregnancy.

The first signs of ADHD generally appear during the first year of life: sleeping disorders (short sleeping and waking to a small noise), spasm of the sob, tremor of the hands and the arms, intense emotionality, frequent tears, hyper-excitability, often fidgets or squirms, doesn't keeps one's seat, inclination to put in danger their life, climbs, doesn't see the danger. Rapidly, the adaptation to the nursery school at three or four years becomes difficult, with hyper-excitability, impulsivity and difficulty to be a good listener.

At six years, all the clinical symptoms of ADHD as described in DMS-IV manual (Findling *et al*, 1997) are present:
- hyperactivity: fidgets with hands or feet, or squirms in seat; gets up from seat when remaining seated is expected; runs about or climbs when it is not appropriate; is "on the go" or often acts as if "driven by a motor"; talks excessively; blurts out answers before questions have been finished; has difficulties in awaiting their turn; interrupts or intrudes on others; has inclination to put their life in danger.
- impulsivity: becomes aggressive, doesn't control their movements; get angry and stops this aggressiveness with difficulty.
- attention deficit: symptoms of inattention should be present for at least six months and when children are at the primary school; does not give close attention to details or makes careless mistakes in schoolwork or other activities; has trouble keeping attention on tasks or play activities; does not follow instructions and fails to finish schoolwork, chores or duty in the work place (not due to opposite behavior or lack of understanding instructions but due to slowness to execute the tasks); has trouble organizing activities; avoids dislikes or does not want to do things that take a lot of mental effort for a long period of time; loses things needed for tasks and activities (toys, pencils, books or tools); is often easily inattentive; is often forgetful in daily activities and has memory difficulties.

In addition, other clinical symptoms occur frequently and must been considered as main symptoms. These include:
- sleeping disorders: mild sleeping problems; cannot put to sleep; has nightmares, broken sleep; is frightened by the night, and awakes weary; very frequent abdominal pains occurring with stress.
- unexplained weariness: weariness begins in the morning with slowness to stand up, to get dressed, and abnormal weariness in sports activities.
- feeling of faintness with or without losing consciousness; twitches in the face or in the breathing reflect hyper-excitability; gnaws one's nails; anxiety, stress, mood disorders, easily upset; loss of trust and loss of respect of oneself; often breakdown; is afraid of people and frightened to die. The medical examination reveals a Chvostek's signs in 66% sign of hyper-excitable children.

In the clinical description of ADHD, biological data are usually not evoked. However, for instance, hyper-excitability can be revealed by an intracellular magnesium deficiency since magnesium was shown to be involved in the control of some nervous system processes.

Clinical Case Study 1
In the first months of her life, patient OCE presents signs of hyper-excitability; she twiddles her fingers, jumps to a lower noise and wakes often. A prescribed calcium therapy was not effective and the tremors persist. But, alternate calcium and magnesium therapy improves all the signs.

At five years, before beginning primary school, patient OCE again presents signs of hyper-excitability; she is fidgety and doesn't stay in her seat; sleeping disorders have reappeared where she awakes very often and sleeps very little; she was impulsive and slaps children, although it was not intentional; she doesn't control herself and was subject to terrible fist of anger. The possibility of magnesium deficiency is raised since her mother has suffered from "spasmophilia" for some years. Both OCE and her mother showed a decrease in erythrocyte magnesium levels.

After two months of Mg^{2+}/vitB6 treatment, OCE feels better. She has become cool, good, less capricious, doesn't play the fool, and her sleeping is calm. Erythrocyte magnesium is increased and the therapy was accordingly stopped. Rapidly, some months later, all the ADHD signs reappeared including attention deficit, hyperactivity, sleeping

disorders and impulsiveness. Biological control confirms the diagnosis of magnesium metabolism disorder (magnesium imbalance) a “genetic disease". The treatment was taken up again with symptoms resolving. However, each time where OCE wants to stop taking the treatment, ADHD symptoms return! OCE received magnesium therapy during eight years: magnesium 6mg/kg/day and vitamin B6 0.6 mg/kg/day (Figure 1).

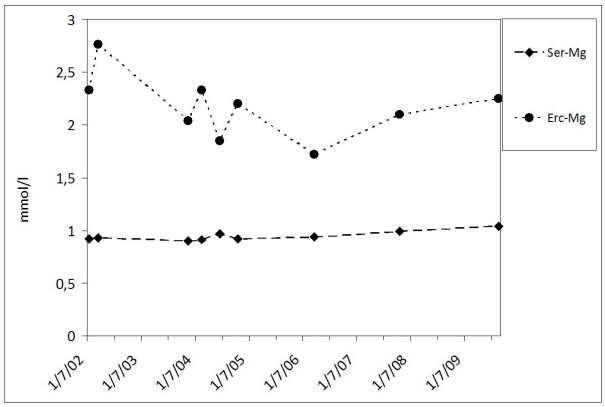


Figure 1. Evolution of biological parameters for magnesium in the case of patient OCE (born February, 1997) during the follow-up (ADHD syndrome). Ser-Mg: total serum magnesium concentrations in mmol/l; Erc-Mg: magnesium concentrations in erythrocytes (obtained after red cell lysis) in mmol/l.

Clinical Case Study 2

Patient NIC was seventeen years old when he came to the consulting office. In the first years of his life, he demonstrated sleeping disorders, a restless sleep, frequent tears, had a delay with communication, was an emotional child, uneasy, over-excitable, and a little aggressive. Upon admission to secondary school, behavioral disorders persisted. He was impulsive, didn’t control himself, was aggressive and also had dyslexia.

A diagnosis of ADHD was made and psycho-stimulant treatment was prescribed over three years. He never took the prescribed dosage because his physician (his grand-father) and his pharmacist had warned his parents of the risks of this medication (methylphenidate) listed in the drug category. Rapidly, he presented side effects of this treatment, including sleeping disorders, muscular pains, weariness, "feeling lethargic", behavioral disorders, "became as a zombie", headaches, palpitations, anxiety attacks, breakdowns, and "was frightened to die". His parents were worried by these side effects, and read our publications at the beginning of 2007. The rates of erythrocyte magnesium, calcium, and ionized calcium had dramatically decreased. The parents decided to stop the psycho-stimulant treatment. A magnesium supplementation (300 mg) spectacularly improved patient NIC's behavior: in some weeks, NIC became cool and more relaxed. An excellent participation in scholarly activities and a better concentration in tasks resulted in excellent scholar results in four months. NIC received the "congratulations of the school-board" at the end of the scholar year 2007! NIC could control himself, was not aggressive, had no behavioral disorders, and had self-confidence. In six months, all the biological disturbances were normalized. At the school of "stone cutter for the historic monuments", NIC has excellent assessments: "good student, hardworker"! NIC received a magnesium supplementation for more than three years and each time when he tried to decrease the dose, clinical symptoms reappeared.

The disease, called "*hypomagnesemia with secondary hypocalcemia*", is heritable. NIC’s sister was also emotional and hypersensitive while his brother was hyper-excitable, always "on the go " and had both sleeping and behavioral disorders. He had magnesium deficiency and now feels better with magnesium therapy. His mother presented with cramps, muscular pains, pins and needles in the hands, stress and migraines. A biological profile showed an erythrocyte magnesium deficiency; after six months of magnesium therapy, the signs disappeared. The maternal grandfather suffers from Alzheimer disease. His father was emotionally stressed, uneasy and for several years experienced sleeping disorders, heart disease, attention deficit, and above all memory disorders. He is only forty years of age! He had an erythrocyte magnesium deficiency and after six months of the same treatment, the memory improved (Figure 2).

In March 2007, the methylphenidate treatment was stopped and the magnesium therapy was set up. A clear behavioral improvement was observed. However, after the three years of magnesium therapy, all biological data remain

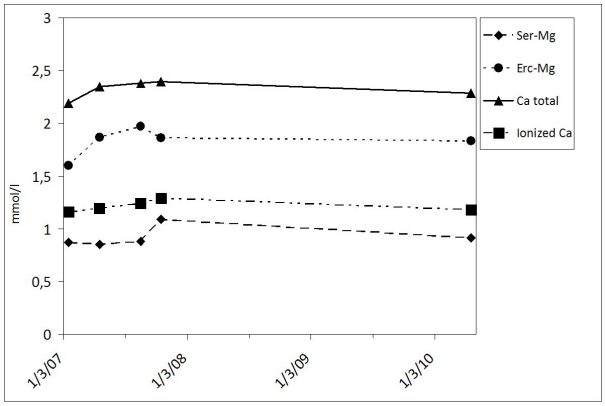


Figure 2. Evolution of biological parameters for magnesium and calcium in the case of patient NIC during the follow-up (ADHD syndrome). Ser-Mg: total serum magnesium concentrations in mmol/l; Erc-Mg: magnesium concentrations in erythrocytes (obtained after red cell lysis) in mmol/l; Total Ca: total serum calcium concentrations in mmol/l; Ionized calcium concentrations in mmol/l in serum.

stable. This result could suggest an alteration of the magnesium transport; it encouraged us to perform additional experiments on magnesium channels in order to check their expression in various tissues.

Experimental Study Number 1

In order to study relationships between hyperactivity symptoms and Erc-Mg levels, we designed an open study on 40 children with ADHD syndrome. For ethical reasons, we chose not to perform a double-blind study against either psychostimulants (methylphenidate) or placebo: parents would not support such a design. In addition, psychostimulants were found to alter magnesium homeostasis (Tolbert *et al*, 1993). Our results (Mousain-Bosc *et al*, 2004) showed a statistically significant improvement of the symptoms after Mg-B6 supplementation, together with a rise in Erc-Mg values.

In this study, a slight but significant intra-erythrocyte Mg^{2+} depletion was evidenced in ADHD patients together with a concomitant decrease in i-Ca concentrations. As we know, Mg^{2+} is essential 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 incidences on neuronal Mg concentrations. The impairment to get 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^+ contents of erythrocytes in sickle cell disease (DMS-IV). Erc-Mg was described as a controversial biological parameter for the monitoring of Mg^{2+} deficiency, in contrast to others (Borella *et al*, 1993; Vink *et al*, 2009) who consider Erc-Mg as a suitable index. Moreover, Basso et al (2000) and Helpern et al (1998) present Erc-Mg as not useful for 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). In any case, in our hands, Erc-Mg measurements were standardized and it appears 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 (Heath and Vink, 1998).

We also found that increased hyperactivity and decreased scholar attention were associated with 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? It was classically accepted that Mg^{2+} is associated with pyridoxine to decrease 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 behavior with hyperactivity was found, psychostimulants are used to improve mental health, probably by increasing synaptic noradrenaline activity. In children who received methylphenidate, a significant increase (6%) in

plasma Mg^{2+} concentrations was found depending on the dosage of the drug, showing a relationship between improvement of hyperactivity and Mg^{2+} metabolism (Schmidt *et al*, 1994 (Tolbert *et al,* 1993). More recently, in autistic children with behavioral disorders and hyperactivity (Zilbovicius *et al*, 2000; Schmidt and Taylor, 1988; Chakraborti *et al*, 2002) positive emission tomography (PET) has shown a significant decrease in cerebral blood flow localized at the temporal lobes level in 76% of the children examined. Taken together with the fact that intra-erythrocyte free Mg^{2+} is associated with increased blood pressure (Zilbovicius *et al,* 2000) and that brain from rats fed with low Mg^{2+} diet are more susceptible to permanent brain focal ischemia (Gervais *et al*, 2004), we can hypothesize that intracellular Mg^{2+} deficiency could be responsible, at least in part, of 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 behavioral abnormalities that is relevant for a Mg-B6 diet. It is evident that another accessible Mg^{2+} store, more significant for central disorders, has to be found.

In conclusion, this study provides 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 intraeythrocyte 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 low scholar attention, beside other traditional therapeutic treatments, a magnesium supplementation could be required in children with ADHD.

Etiology and risk factors for ADHD

The etiology of ADHD is not yet known. The causes are multifactorial:

- genetics: twin studies indicate that heritability ranges from 60 % to 90 %; various genes are currently being studied (magnesium is involved in gene expression and essential for membrane stability) (Ozgo *et al*, 2007).
- brain abnormalities: it is believed that the dopaminergic system is involved in ADHD. Magnesium has an impact by reducing the release of neurotransmitters and other mediators. Magnesium also stabilizes the membranes (Nemoto *et al*, 2006).
- environmental and peri-natal factors: studies in rats suggest that a magnesium-deficient diet influences not only mineral metabolism but also protein metabolism. Growth retardation results from the low food intake that is induced by magnesium deficiency, which provokes an alteration in energy metabolism. But the protein nutritional status in magnesium-deficient rats is restored by dietary magnesium supplementation in seven days. In women, maternal magnesium intake has an immediate effect on placental vascular flow. Magnesium sulphate reduces the vasoconstriction effect of angiotensin II in human placenta. Reduced placental vascular flow is at least, in part, responsible of placental insufficiency and intrauterine growth retardation. Magnesium deficiency increases the risks of miscarriage symptoms, premature delivery, fetal growth retardation, twin pregnancy, and stress (due illness/depression leading to a loss of magnesium by the kidneys, and increased magnesium requirements); thyroid dysfunction (Takaya *et al*, 2006).
- essential fatty acid imbalance: nutritional factors such as essential fatty-acid (EFA) deficiencies have been associated with ADHD. The principal omega-3 fatty-acid in the brain, DHA, is highly accumulated in nervous tissue membranes and is important for neural function. Studies of diet showed that children with ADHD consumed equal amounts of omega-3 and omega-6 fatty-acid, compared to control children. However, ADHD children had significantly lower levels of DHA and total omega-3 fatty-acids, higher omega-6 fatty-acids, and lower ratios of omega-3/omega-6 fatty-acids, compared to control children. These results suggest that adolescents with ADHD have abnormal EFA profiles, which are not explained by

differences in intake. The role of magnesium will be evoked in the hypothesis of imbalance of essential fatty-acids. Vitamin B6 is also an important cofactor for numerous metabolic reactions including metabolism of serotonin, GABA (gamma-amino-butyric acid) and dopamine (Colter *et al,* 2008, Gonon, 2009).

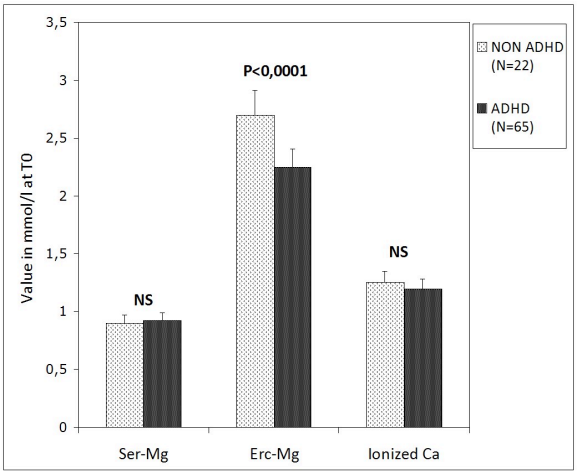


Figure 3. Biological data obtained at the first visit for 65 ADHD and 22 non-ADHD children. Ser-Mg: total serum magnesium concentrations in mmol/l; Erc-Mg: magnesium concentrations in erythrocyte (obtained after red cell lysis) in mmol/l. Only Erc-Mg appeared to be significantly lower than before treatment (statistically significant at $p<0.05$).

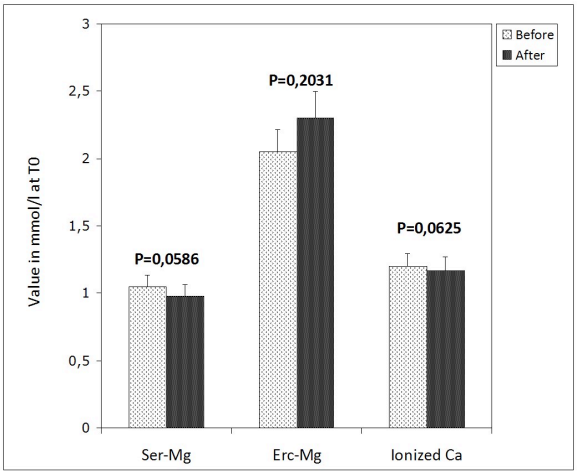


Figure 4. Biological data obtained 3 months after the first visit for the same children. Ser-Mg: total serum magnesium concentrations in mmol/l; Erc-Mg : magnesium concentrations in erythrocyte (obtained after red cell lysis) (in mmol/l). Erc-Mg recovered normal values (statistically significant at $p<0.05$).

Treatment of ADHD

Conventional therapy is often multimodal including behavioral therapies and medications. Approved drugs for ADHD are psychostimulants (amphetamine derivatives), including methyl-phenidate. Psychostimulant medications approved by the U.S. Food and Drug Administration (FDA) include methylphenidate (Ritalin^{R}, Concerta^{R}), and, more recently, atomoxetine (Strattera^{R}).

In 2008, a study by the National Center of Scientific Research (CNRS) in France (Gonon, 2009) concluded that "*although psychostimulants alleviate the core symptoms of attention deficit hyperactivity disorder (ADHD), recent studies confirm that their impact on the long-term outcomes of ADHD children is null. Psychostimulants enhance extracellular dop-amine*". Numerous review articles assert that they correct an underlying dopaminergic deficit of genetic origin. This dopamine-deficit theory of ADHD is often based upon an overly simplistic dopaminergic theory of reward. Psychostimulant medication does not improve long-term academic outcomes of the ADHD children. Therefore, this hypothesis should not be put forward to bias ADHD management towards psychostimulants. The American Journal of Psychiatry in June 2009 published a study "Sudden death and use of Stimulant Medications in Youths". This study was funded, in part, by the FDA and the National Institute for Mental Health (Gould *et al*, 2009). Side effects are described for psychostimulant medications over some years.

As we have seen previously, dietary factors and magnesium deficiency can play a significant role in the etiology of ADHD syndrome. In our study, magnesium deficiency was found in 89% of children with ADHD, whereas only 23% of non-ADHD children present a low erythrocyte Mg^{2+} deficiency (Table1).

Clinical symptoms of autistic spectrum disorders (ASD)

The prevalence of autism increases continuously. It remains an extreme challenge to clinical researchers. It is a neurobiological condition having its origin in a disturbance of the cellular structure of the brain during pregnancy. Generally speaking, there is no "cure" for such children and

Table 1.

Value in mmol/l		Non ADHD (N=22)	ADHD (N=85)
Threshold value		% children < threshold	
Ser-Mg	0.85	0.00	27.69
Erc-Mg	2.46	22.73	81.54
Ionized Ca	1.18	0.00	19.05
At least one criterion under threshold		23.81 (5/21)	88.89 (56/63)

the holy grail of finding a neuropharmacological reversal of symptoms is currently being researched worldwide.

For some years, one must insist on the screening of the early signs of autism during the two first years of the life as described in DMS-IV manual:

- Relationship disorders; too good child with very few smiles.
- Visual contact disorders; missing or poor visual attention; doesn't look at the parents; look is shifty.
- Difficulty in listening; seems not to hear; delayed communication; doesn't repeat words.
- Motor affectation; doesn't take the toys; stereotyped movements of the hands; sits and walks belatedly; hypo-tonicity or hyper-tonicity.
- Sleeping disorders; broken sleep; crying.
- Feeding difficulties; slobbers; cannot swallow; refuses food.

The major feature of autism spectrum disorder is that they only manifest themselves after the age of eighteen months and before the age of three. it is considered like a regression of the development (Burn, 2009). So, we can explain that ASD are disorders of brain function and not of brain structure.

After three years, the clinical picture of ASD becomes typical:

- Impairment of social interactions:
  - visual contact: doesn't look at the parents and the siblings; gives a blank look; look is shifty
  - connection with equals: has no interest with the parents, with the friends at the primary school; seems to be "in their bubble"
  - delight partitions: has no emotional relation with the family, cannot express their pleasure for the event. Social reciprocity: has no answer to a human presence.
- Loss of communication:
  - delayed communication; speaks belatedly; some sounds or some syllables are emitted late; few spontaneous verbal exchanges; lack of creativity in thought.
  - no communication: doesn't repeat words.
  - Stereotypical language: repeats the same word sometimes without significance.
  - social mimicking: cannot reproduce something after a long time earnestly trying.
- Stereotyped restricted behavior:
  - stereotyped interest: he has very poor interest or repeats the same answer for something.
  - customs.
  - handling things: difficulty to take the things, to take toys, and can have abnormal movements.
  - motor affectation: to walk, moves with difficulty; to bring something to somebody, cannot take initiative.
- Abnormal or delayed functioning:
  - language.
  - social interactions.
  - symbolic games: doesn't understand the rules of the game; cannot dream up the game.
  - behavioral disorders like sleeping disorders, aggressiveness, impulsivity; attention deficit cannot listen.

Magnesium metabolism could be involved in autism, autism spectrum disorder or pervasive developmental disorder (Rimland *et al*, 1978): magnesium prevents against encephalopathy and developmental delay (Doyle *et al*, 2008).

Clinical Case Study 3

Patient ARN, three years old, presents all the clinical signs of ASD (Autistic Symptoms Disorder), an autistic disorder that began after two years. The adaptation at the nursery school became difficult. His mother describes behavioral disorders, with incessant restlessness, doesn't listen, puts own life in danger; in the street, let's go the mother's hand; crosses the street without looking around; has important sleeping and communication disorders; he doesn't speak, is always squealing; his glance is shifty. At school, the headmaster's evidence was spectacular: "ARN become isolated, doesn't draw, has no interest with the activities, doesn't listen about the forbidden subjects. He doesn't speak, expresses himself by shouting, his glance is shifty". During the examination, patient ARN was nervous, restless, tetanized, he clench's his fists and shakes h.is hands. In front of all these clinical symptoms, the possibility of a magnesium deficiency is raised; his mother has presented a magnesium deficiency for several years.

With a Mg^{2+}/vitB6 therapy, (6 mg/kg/day of magnesium and 0.6 mg/kg/day of vitamin B6), ARN improved quickly month after month, probably because magnesium supplementation was set up before the age of four years, very early in life. ARN becomes cool, obedient, wants to get dressed alone, put his shoes on, takes interest in the family activities, is looking to manipulate the computer, begins drawing, doesn't cry, says some words and some sentences. At school, he begins to participate in the games, takes a book, shows the pictures and repeats the words. The improvements are spectacular. After six months treatment, he can pronounce "complete sentences" and draws. After eighteen months therapy, the parents stopped therapy, however the behavioral disorders reappeared with incessant restlessness at home and at school in two weeks. Another attempt to stop the treatment was also unsuccessful where he became tired and depressed. The parents now do not want to stop the treatment anymore.

Today, patient ARN is twelve, doesn't present a behavioral disorder and communication and language is almost normal. He begins high school and has taken Mg^{2+}/vitB6 supplementation for eight years (additional biological data: karyotype is 46 XY; fragile X negative).

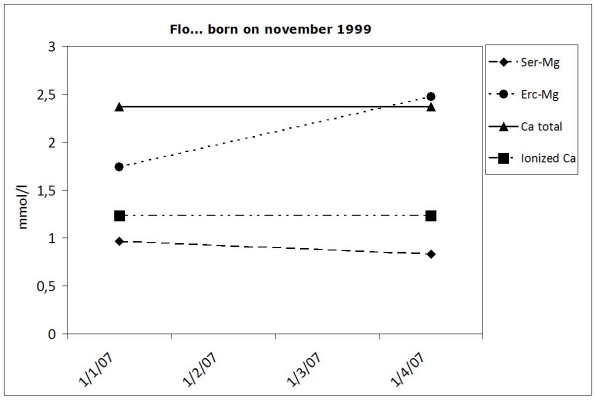
Clinical Case Study 4

Patient FLO was four years old when the diagnosis of "typical autism" was made. When he was seven years old, the parents were informed about our work on magnesium and autism. He presented communication disorders, could not emit sounds, didn't talk, had abnormal visual reactions, didn't look someone in the eyes, had a blank look and his look was shifty. The behavioral disorders were important with many anxiety attacks, difficulty to calm, impulsiveness and always moving with fits of anger. He had no creativity for the games and didn't know how to play. Erythrocyte magnesium was low. The same observation was made in his parents (Figures 5-7), despite them having a balanced diet including fishes, meats, fruits and vegetables.

After two months treatment, (magnesium 6 mg/kg/day and vitamin B6 0.6 mg/kg/day), patient FLO was more cool, stayed at school with pleasure, began to talk with little sentences, was less in "his bubble", and was looking for his father when he came back from work. He was beginning to play with friends. After three years of treatment, FLO talks appropriately, understands the language, goes to a normal school where reading skills have developed. During the first discussion, the parents advise that they want a third child. They both have evidence of important magnesium depletion. A preventive magnesium supplementation both before the desired pregnancy and during the pregnancy seems to be essential, and indeed allowed good development of the baby without baby blues in the mother. The little sister is now two years old, bright, very much smiling and is always in a good mood! (Additional biological data: search for mutation in the gene neuroligine NLGN3 was negative; search for mutation in the gene neuroligine NLGN 4 X was also negative).

Experimental Study Number 2

In order to study the effect of Mg-B6 for treating social, communication and behavioral responses of children with pervasive developmental disorders (PDD) or autism in connection with the designed an open study on 33 children with PDD magnesium/calcium status of the child, we



Figures 5. Evolution of biological parameters for magnesium and calcium in the case of patient FLO (born November 1999) during the follow-up (ASD/PED syndrome). Ser-Mg: total serum magnesium concentrations in mmol/l; Erc-Mg: magnesium concentrations in erythrocytes (obtained after red cell lysis) in mmol/l; Total Ca: total serum calcium concentrations in mmol/l; Ionized calcium concentrations in mmol/l in serum.

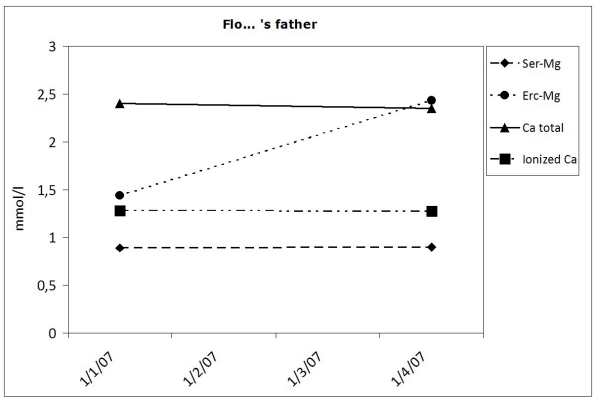


Figure 6. Evolution of biological parameters for magnesium and calcium in the case of patient FLO's father during the follow-up. Ser-Mg: total serum magnesium concentrations in mmol/l; Erc-Mg: magnesium concentrations in erythrocytes (obtained after red cell lysis) in mmol/l; Total Ca: total serum calcium concentrations in mmol/l; Ionized calcium concentrations in mmol/l in serum.

designed an open study on 33 children with PDD syndrome. Our results showed a statistically significant improvement of the symptoms after Mg-B6 supplementation together with a rise in Erc-Mg values (Mousain-Bosc *et al*, 2006).

Intraerythrocyte Mg^{2+} (Erc-Mg), serum Mg^{2+} (s-Mg) and blood ionized Ca^{2+} (i-Ca) were measured

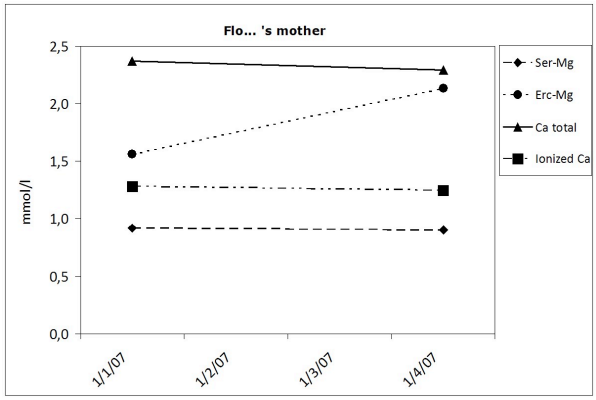


Figure 7. Evolution of biological parameters for magnesium and calcium in the case of patient FLO's mother during the follow-up. Ser-Mg: total serum magnesium concentrations in mmol/l; Erc-Mg: magnesium concentrations in erythrocytes (obtained after red cell lysis) in mmol/l; Total Ca: total serum calcium concentrations in mmol/l; Ionized calcium concentrations in mmol/l in serum.

at different times. Clinical symptoms of PDD were scored (0 to 4). In contrast to s-Mg or i-Ca, PDD children exhibited significantly lower Erc-Mg values than controls (1.26 times; 16/33). The Mg-B6 regimen led to an increase in Erc-Mg values (1.18 times, 11/17) and this supplementation improved PDD symptoms in a large majority of children with no adverse effects: social interactions (23/33, $p<0.0001$), communication (24/33, $p<0.0001$), stereotypical restricted behavior (18/33, $p<0.0001$), and abnormal/ delayed functioning (17/33, $p<0.0001$); 15/33 children improved in the first three groups of symptoms. When the Mg-B6 treatment was stopped, PDD symptoms reappeared in a few weeks. A statistically significant relationship was found in Erc-Mg values from children before treatment and their mothers.

The neurobiological basis of a Mg^{2+}/vit B6 supplementation supposes the existence of an impaired neuronal Mg^{2+} pathway which could be reversed with Mg-B6 therapy. As we have previously discussed, Mg^{2+} acts as an ionic membrane regulator and modulator of ion transfer through membrane channels. In brain, it has been shown that traumatic injury causes a decline in Mg^{2+} concentrations, focally as well as in blood circulation, and contributes to the development of neurologic deficit (Vink *et al,*

2009). Similarly, brain ischemia caused a decline in intracellular free Mg^{2+} concentrations (Basso *et al,* 2000) and magnesium salt administration improves motor outcome in this situation (Ebel and Gunther, 2005). One of its most important modes of action is to inhibit the glutamate N-methyl-D-aspartate (NMDA) channel (Zilbovicius *et al,* 2000). The activity of this channel generates an influx of calcium and, in turn, leads to excitotoxic cell death and apoptosis (Borella *et al,* 1993). In the same way, abnormal dietary deficiency of Mg^{2+} as well as abnormalities in Mg^{2+} metabolism play important roles in different types of heart diseases, and Mg^{2+} influences catecholamine signalling in such diseases (Gervais *et al*, 2004).

Recently, in primary autistic children, positive emission tomography (PET) has been used to demonstrate a significant decrease in cerebral blood flow localized to the temporal lobes in 16/21 of children (Zilbovicius *et al*, 2000; Macdonald *et al*, 2004; Demougeot *et al,* 2004). Taken together with the fact that Mg^{2+} has been shown to increase blood pressure (Macdonald *et al*, 2004) and that brain from rats fed with low Mg^{2+} diet are more susceptible to permanent brain focal ischemia (Demougeot *et al*, 2004), we can hypothesize that intracellular Mg^{2+} depletion could be responsible, at least in part, of some central activity disorders observed in PDD/autistic children.

In our study, an intra-erythrocyte Mg^{2+} depletion was evidenced in almost half of the PDD children. To explain such a phenomenon, two hypotheses can be proposed: (i) a metabolic inhibition of membrane Na^+/K^+ ATPase (observed in autism (Kurup and Kurup, 2003) with concomitant rise in intracellular Ca^{2+} and decrease in intracellular Mg^{2+}; (ii) a genetic defect in magnesium transport through plasma membrane (Na^+-Mg^{2+} exchanger (Ebel et al, 2004; Ebel and Gunther, 2005) or TRPM chanzymes (Montell, 2005). As Erc-Mg can be considered as representative of some intracellular Mg concentrations, a decrease in Erc-Mg without changes in serum Mg concentration could be interpreted as an alteration of Mg^{2+} transport through the plasma membrane. The demonstration that TRPM7 is critical for Mg^{2+} homeostasis evoked the possibility that mutation of TRPM channels may cause disease in humans as a result of reduced intracellular Mg^{2+} levels. Indeed, mutations were found in the case of hypomagnesemia with secondary hypocalcemia disease (Schlingmann *et al,* 2008) and, in this case, symptoms associated with TRPM6 mutations were improved by supplementation with high Mg^{2+} doses, in agreement with increased Mg^{2+} entry through a passive mode of Mg^{2+} influx. This genetic hypothesis was also supported by our own data showing a positive correlation between low Erc-Mg values in PDD children and their mothers. Similarly, Feillet-Coudray et al (2006) have found that, in mice genetically selected for low magnesium levels, Mg efflux from erythrocytes was significantly increased. The genetic regulation of erythrocyte Mg^{2+} content depends on the modification of Mg^{2+} influx (Schlingmann *et al,* 2002). To confirm such a hypothesis, a genetic study of PDD children's family has clearly to be developed.

When PDD children were supplemented with Mg-B6 treatment, Erc-Mg values more or less increased in only 65% of children. The impairment to get normal Erc-Mg values under Mg-B6 treatment supports the hypothesis of a defect in Mg^{2+} transport in erythrocytes. In sickle cell disease, Mg pidolate supplementation was found to decrease Na^+/Mg^{2+} exchanger activity with a partial rise in Mg^{2+} and K^+ contents of erythrocytes (De Franceschi *et al*, 2000). Doses of Mg-B6 and the duration of treatment, which have not been taken into account in our study, could also explain such an observation. Concerning the respective roles of pyridoxine and Mg^{2+} in these observations, it was classically accepted that Mg^{2+} is associated with pyridoxine to decrease irritable side-effects of the B6 therapy and that B6 is the main factor involved in the improvement of clinical symptoms in autistic patients. Following Erc-Mg values during Mg-B6 treatment, we bring here evidence of the role of Mg^{2+} itself in this therapy.

Mg-B6 treatment of PDD children was shown to improve symptoms of the disease. Three of the four main groups of clinical signs described in DSM-IV were significantly reduced and we found for the first time that 8/12 of children who improved under treatment showed higher Erc-Mg values. Persons only slightly deficient in magnesium become irritable, high-strung, sensitive to noise, hyperexcitable, apprehensive

and beligerent. If the deficiency is more severe or prolonged, they may develop twitching, tremors, irregular pulse, insomnia, muscle weakness, jerkiness, and leg and foot cramps. These symptoms can also be found in some cases of PDD/autism. Although this study was an open non-controlled study, we found a relationship between clinical signs of PDD/autism and a biological parameter, namely Erc-Mg. However, we were unable to establish any correlation between improvement of symptoms and increase in Erc-Mg. Various possibilities can explain this lack of correlation: (i) Erc-Mg is probably not the best biological parameter to follow the relationship between magnesium homeostasis and neurological dysfunctions of PDD/autism. Contradictory reports have been published on the use of Erc-Mg as index of Mg^{2+} status (Borella *et al*, 1993, Basso *et al*, 2000) and new biological tests that could help to study genetic alterations of magnesium transport (lymphocytes, etc) have to be tested. (ii) Other neurofunctional disorders may be involved in autism, such as a decrease in temporal blood flow. Even if low Erc-Mg levels have been shown to be related to decrease in blood pressure, there is no evidence to associate blood pressure and cerebral blood flow in all cases.

In conclusion, this study brings new information about the therapeutic role of a Mg-B6 regimen in children with PDD syndrome. This effect seems to be associated, at least in part, to a cellular Mg^{2+} depletion as evidenced by intraeythrocyte Mg^{2+} measurements. Children with pervasive developmental disorders (including autism) exhibit low Erc-Mg levels. Parents frequently showed similar low Erc-Mg values suggesting a genetic defect in Mg^{2+} transport. Installing a Mg-B6 supplementation for some weeks restored higher intraerythrocyte Mg^{2+} values and significantly reduced the clinical symptoms of these diseases.

Etiology: role of magnesium during pregnancy

Since developmental disorders appear early during fetal development, magnesium therapy could be justified even during pregnancy. The efficacy of magnesium supplementation is more important if treatment is in the early years of life. Magnesium activates protein synthesis and amino acid synthesis and magnesium deficiency leads to fetal growth retardation by reducing the nutritive utilization of protein as a result of decreased protein absorption and synthesis. Another study found a low cerebral blood flow in the temporal lobe probably due to a low magnesium level in the cells. (Zilbovicius *et al*, 2000)

Effect of magnesium in fragile-X syndrome

Fragile-X syndrome is an X-linked disorder characterized primarily by speech delay and moderate mental retardation and neurobehavioral disorders. The incidence of fragile X syndrome is estimated at 1/4000-1/6000 males and half that for females (Wiesner *et al*, 2004). This disease is linked to the mutation of the FMR1 gene located on the X chromosome, characterized by expansion (CGG amplification) at the FRAXA site (Xq27.3) in the non coding region of the first exon (Weisman-Shomer *et al*, 2002). In 2002, Weisman Shomer revealed two factors indispensable to the stabilization of the CGG tetraplex : magnesium and ATP.

We report a single case of a child referred to our pediatrics unit for a behavioral disorder and for whom Fragile X syndrome had previously been genetically confirmed. Considering the genetic origin of this disease, our work was completed by the clinical and biological evaluation of the patient's family. Both the patient and his affected family members were given a magnesium and vitamin B6 supplementation and evaluated over three years. A couple of family, including children with mental retardation, pervasive development disorder or attention deficit hyperactivity disorder, were followed clinically and biologically for three years. All children carried a mutation produced by expansion at the FRAXA site at Xq 27.3 and showed magnesium and calcium disorders (normal serum Mg^{2+} concentration, but decreased erythrocyte Mg^{2+} and plasma ionized Ca^{2+}). The mother presented a premutation by expansion at the FRAXA site at Xq 27.3 with symptoms of magnesium depletion (emotivity, asthenia, stress). Mg^{2+}/vitB6 supplementation (magnesium 6mg/kg/day and vitamin B6 0.6 mg/kg/day, orally) for three years reduced clinical symptoms in the mother and improved the behavior of the children (aggressiveness, lack of attention at school) concomitant with an increase of intraerythrocyte Mg^{2+}. When the Mg^{2+}/vitB6

treatment was stopped for two months in one of the children, clinical symptoms reappeared.

In 2007, the hypothesis was put forward that Fragile X syndrome (Fra-X) is caused by the transcriptional silencing of the FMR1 gene that encodes the Fra-X mental retardation protein (FMRP) (Dolen *et al*, 2007; Hayashi *et al*, 2007). However, the pathogenesis of this disease is unknown. According to one proposal, many psychiatric and neurological symptoms of Fra-X result from the unchecked activation of mGluR5, a metabotropic glutamate receptor. To test this hypothesis, FMR1 mutant mice presenting a 50% reduction in mGluR5 expression were generated and studied in terms of the range of phenotypes with relevance to the human disorder. Results demonstrated that mGluR5 contributes significantly to the pathogenesis of the disease, a finding that has significant therapeutic implications for Fragile X and related developmental disorders. In line with this observation, Hou et al (2006) demonstrated that mGluR-LTD induces a transient, translation-dependent increase in FMRP that is rapidly degraded by the ubiquitin-proteasome pathway.

The successful management of this disease by magnesium and vitamin B6 supplementation observed in the present study, with an early and late evaluation at 3 years, supports the argument that a deficit in Mg^{2+} influences the appearance of Fra-X and that by replacing this magnesium, the disease symptoms may be managed. Berthelot's group (Martin *et al,* 2007) has previously demonstrated in rats that a deficit in magnesium acts by reducing the level of proteasomes, protein complexes which act at the level of the mGluR5 receptor already implicated in this disease. Conversely, Picado (Picado *et al*, 1994) noted an increase in the levels of Mg^{2+}/NA in patients with behavioral problems and for whom hypertension was diagnosed. It has been demonstrated that for behavioral disorders in which hyperactivity and autism in particular are involved, for which current treatment regimens are difficult to standardize, management of disease symptoms with magnesium and vitamin B6 supplementation is possible (Mousain-Bosc *et al*, 2006). Magnesium depletion represents one possible explanation for the appearance of Fra-X syndrome. Studies show that magnesium-vitamin B6 supplementation given to subjects improved their behavioral disorders. The presence of a genetic mutation together with magnesium deficiency in mothers may constitute an indication for regular evaluation of magnesium status and Mg^{2+}/vitB6 supplementation during pregnancy. We present herein some unpublished data obtained with 3 cases of Fra-X abnormality (poster presentation in Gordon Research Conference, Ventura, CA, March 2008).

Conclusions

This review brings additional information about the therapeutic role of a Mg^{2+}/vitB6 regimen in children with ADHD or ASD/autism syndrome. This effect seems to be associated, at least in part, to a cellular Mg^{2+} depletion as evidenced by intraeythrocyte Mg^{2+} measurements. Children with ADHD or PDD/ASD (pervasive developmental disorders/autistic spectrum disorders), including autism, exhibit low Erc-Mg levels. Parents frequently showed similar low Erc-Mg values suggesting a genetic defect in Mg^{2+} transport. Installing a Mg^{2+}/vitB6 supplement-ation for some weeks restored higher intraerythrocyte Mg^{2+} values and significantly reduced the clinical symptoms of these diseases.

However, in a recent meta-analysis of all studies, Nye et al (2010) arrived at the same conclusion as what they published in 2005: "*Due to the small number of studies, the methodological quality of studies, no recommendation can be advanced regarding the use of B6-Mg as a treatment for autism. There is simply not sufficient evidence to demonstrate treatment efficacy*". Together with the fact that both the American Psychiatric Association and the American Academy of Pediatrics have stated that megavitamin treatment for learning disabilities and autism is not justified, it helps explain the dearth of clinical studies investigating the use of magnesium and vitamin B6 (Mg-B6) in the treatment of autism and autistic spectrum disorders.

Magnesium is known to be crucial for brain activity and its involvement in the prevention of neurobehavioral diseases seems to be established. As a clinical double-blind study with Mg^{2+}/vitB6 treatment over placebo cannot be accepted for regulatory and ethical reasons, it was suggested to put children under an "alternative treatment" before the conventional

drug therapy. We hope that the combined use of new tools to measure intracerebral magnesium levels as proposed by the group of Iotti et al (2008) and a more specific clinical evaluation will help to improve the outcome of children with these pathologies.

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