Zinc therapy of neurological Wilson’s disease in a woman with two foetuses with agenesis of the corpus callosum

Tratamento pelo zinco na fase neurológica da doença de Wilson numa mulher com dois fetos com agenesia do corpo caloso

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ABSTRACT | CLINICAL REPORT: A patient diagnosed Wilson’s disease (WD) 22 years previously, successfully treated initially with zinc, developed neuropsychiatric disease after years of irregular therapy. Reassumming zinc therapy was successful. After a normal pregnancy, she had two therapeutic abortions for corpus callosum agenesis, and a missed abortion.

We review the genetics, physiopathology, clinics and imagiologic response to zinc therapy, the problems of pregnancy in WD, advising to maintain therapy. A hypothetic cause for fetus brain anomaly would be hypocupremia due to zinc therapy, confronting with two other possibilities, one related to Wilson’s disease in itself, other due to a congenital syndrome of agenesis of the corpus callosum, impossible to diagnose by our available diagnostic methods. GE–J Port Gastrenterol 2010;17:116-125.

KEYWORDS: Wilson’s disease, zinc, corpus callosum agenesis, hypocupremia.

RESUMO | CASO CLÍNICO: Doente com doença de Wilson (DW) diagnosticada 22 anos antes, tratada inicialmente com sucesso com zinco, desenvolveu doença neuropsiquiátrica após anos de terapêutica irregular. A reintrodução do zinco foi eficaz. Após gravidez normal, teve duas interrupções terapêuticas por agenésias do corpo caloso fetal, e um caso de morte fetal.

Fez-se revisão da genética, fisiopatologia, resposta clínica e imagiológica à terapêutica com zinco, problemas da gravidez na DW, aconselhando-se manutenção terapêutica. Aventa-se hipótese da hipocuprémia induzida pelo zinco ser causal na anomalia fetal cerebral, em contraponto a outras duas possibilidades, uma relacionada com a própria DW, a outra a de ser resultante de uma das várias síndromes congénitas de agenésia do corpo caloso, não diagnosticáveis pelos meios de diagnóstico acessíveis aos autores. GE–J Port Gastrenterol 2010;17:116-125.

PALAVRAS-CHAVE: Doença de Wilson, zinco, agenesia do corpo caloso, hipocuprémia.

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INTRODUCTION

Progressive hepatolenticular degeneration of Wilson’s disease (WD) is an inborn autosomal recessive disorder of copper metabolism, characterised by an inadequate excretion of absorbed dietary copper via bile, resulting in excessive accumulation of copper in the liver, central nervous system, and other organs, that can present clinically with hepatic, neurological or psychiatric disturbances, or a combination of these. Kayser-Fleischer rings result from copper deposition in Descemet’s membrane of the cornea. Mild or acute hemolysis may be a presenting feature. The most important determinant in WD symptomatology and its evolution appears to be the concentration of free copper in serum.

The genetic defect in WD occurs on chromosome 13 at the level of ATP7B gene, which is 80 kilobases in length, consists of 21 exons and gives rise to a 165 kDa membrane bound protein (adenosine triphosphatase ATPase) with 1465 amino acids, with eight hydrophobic transmembrane sequences and six copper binding domains, a transduction domain, a cation channel and phosphorylation domain, and a nucleotide-binding domain. The ATP7B protein is expressed primarily in liver but also in kidney, stomach, spleen, and other tissues. As the typical daily intake of copper (0.6-1.6 mg a day) is more than the human body needs, a well functioning excretion must exist, in order to keep the organism from copper overindulgence leading to toxic effects. This is solved by the liver ATP7B protein, through incorporation of copper into ceruloplasmin for release to the circulation, but also necessary for excreting copper into bile. The exact mechanism of this process is unknown, but ATP7B protein appears to move to the canalicular membrane. At January 2006, around 300 mutations have been described in the ATP7B gene, recorded in the Wilson’s disease mutation database (http://www.medicalgenetics.med.ualberta.ca/wilson/index.php). Most patients are compound heterozygotes, partially explaining the variable spectrum of the disease. Molecular genetic testing of the ATP7B gene is nowadays clinically available and is playing an increasingly important role in diagnosis, as copper studies are frequently equivocal, being sometimes essential for determining the genetic status of at risk sibs.

The mutations cause a decrease or absence of the gene product or production of a protein with impaired function. This results in reduced copper transport within the hepatocyte and failed insertion of copper into ceruloplasmin. As a consequence, in most patients with WD serum ceruloplasmin and total copper serum concentrations are low, but there is an increased proportion of free low molecular weight copper species in serum, which deposits in excess in liver, brain, eyes, and other tissues. Absence of the protein in kidney impairs tubular reabsorption of filtered copper with resultant increased copper urinary excretion.

According to the old paradigm copper accumulation would be noxious, causing symptoms due to organ dysfunction, but today it is accepted that symptoms are caused by copper poisoning due to increased free copper concentration in serum. The accumulation of copper in the liver can be understood as a sign of detoxification of free copper in the liver by sequestration in a non-toxic metallothionein-bound form. If treatment aimed at lowering free copper concentrations in blood is started early, and if treatment is monitored correctly to ensure that free copper concentrations normalize, the symptoms may be completely reversible. If treatment is started later, severe liver disease and severe cerebral lesions may become irreversible.

Today it is generally accepted by several authors that treatment of copper poisoning with chelating agents is contraindicated, because it may lead to an increase in serum levels of free copper and iatrogenic clinical deterioration, sometimes irreversible. Therapy with tetrathiomolybdate is yet it is in a clinical experimental phase, being mostly useful for the treatment of severe neurological cases. Contrarywise to chelator therapy, zinc does not cause neurological deterioration. Some authors utilize zinc acetate 50 mg three times daily whereas others prescribe zinc sulphate 200 mg three times daily. Zinc shall be taken 1 hour before or after meals. Zinc acts by blocking absorption of copper through induction of mucosal intestinal cell metallothionein. Metallothionein has a greater affinity for copper than for zinc, and the metallothionein-copper complex is lost in stool after sloughing of intestinal epithelial cells. Zinc also induces metallothionein synthesis at the liver. Brewer, et al demonstrated that no hepatic deterioration occurred in presymptomatic WD patients treated with zinc and followed for 3 to 9 years.

Copper is deposited throughout nearly all areas of gray and white matter of the central nervous system (CNS), but morphological changes are localized especially in the basal ganglia, deep cortical layers, cerebellum, brainstem and cerebral cortex. MRI is more sensitive than CT to demonstrate the CNS lesions, particularly increased signal intensity on T2-weighted-images (T2WI) over the thalamus, basal ganglia and brainstem, especially the midbrain and pons. Regression of T2WI signal abnormalities on follow-up brain MRIs have been documented in several patients with WD on chelation treatment (D-penicillamine and trientine) and after liver transplantation. Changes in MRI appropriate to clinical worsening or improvement on chelation treatment support copper redistribution either into or out of the CNS. On serial MRI studies along 24 months of continuous treatment with zinc sulphate, Huang, et al demonstrated gradual resolution of the increased signal intensities in accordance with the clinical amelioration of the patients.

There are no contraindications to pregnancy in WD during maintenance therapy, with the exception of the presence of
severe liver disease\textsuperscript{21}, and anti-copper agents shall be continued during pregnancy. Otherwise women can suffer severe regression of their disease, often ending in death. Although some authors\textsuperscript{22,23} report favourable results of treatment of pregnant patients with WD, other authors refer to the possibility of occurrence of serious side effects of penicillamine\textsuperscript{24}, favouring treatment with zinc. Also teratogeny has been documented in experimental animals and sometimes in humans with the chelating drugs penicillamine and trientine, the major factor in its production appears to be copper deficiency in the foetus\textsuperscript{25,26}. Tetrathiomolybdate is not advised in pregnancy. According to several authors zinc therapy is considered essentially non toxic\textsuperscript{27-30}. However Buamah, et al\textsuperscript{31} reports that low serum copper concentrations in pregnant woman during midgestation is a risk factor for foetal anencephaly, and Brewer, et al\textsuperscript{21} documented one case of foetal microencephaly and another of a newborn with minor surgically correctable heart defect in patients on treatment with zinc sulphate.

The authors present a case of a 38-year-old woman that, at the age of 16, was diagnosed a hepatic form of WD\textsuperscript{32}, responding favourably to zinc sulphate therapy during five years, but with neurological deterioration after irregular therapy for several years, and with clinical and imagiological amelioration after reassuring regular therapy with zinc sulphate. With therapy compliance the patient had one normal pregnancy and posteriorly two pregnancy terminations due to brain malformation (agenesis of the corpus callosum associated to ventriculomegaly). A possible correlation of these malformations with hypocupremia caused by zinc therapy is hypothesized. After that she had a missed abortion at 11 weeks of gestation, possibly related to Wilson’s disease. Afterwards she decided to proceed to bilateral tubal ligation.

**CLINICAL CASE**

At 1986 a 16-year old portuguese girl was referred to us from the Hospital of Évora (Alentejo district) with a suspected diagnosis of chronic active hepatitis and hemolytic anemia. A diagnosis of hepatic form of WD was established based on clinical data, Kayser-Fleischer rings, abnormal liver function tests, low serum caeruleoplasmin, hypocupremia and hypercupruria (Table 1); the liver biopsy showed signs of chronic active hepatitis, liver fibrosis and nodular regeneration, and elevated concentration of copper on liver tissue: 536 µg/g of dry liver (N < 250 µg/g of dry liver). There were no neurologic symptoms and neurologic examination was normal. Central nervous system imaging was not performed. There were no other known cases of WD in her family. The patient was treated from then on with zinc sulphate (monohydrated salt) 200 mg three times daily. Five months later the patient was feeling much better and laboratory tests of copper biochemistry also showed favourable evolution (Table 1). One year after initiating the therapy the patient was asymptomatic, Kayser-Fleischer rings disappeared, liver function tests and also copper biochemistry were normal. Liver biopsy showed not only frank improvement of the inflammatory component, but also an almost normal concentration of copper on liver tissue (272 µg/g of dry liver). She was advised to maintain zinc therapy for life. During the next five years she did the therapy regularly, feeling well and being completely asymptomatic. Unfortunately the patient missed the appointments between 1991 and 1999.
taking the medicine irregularly. In 1999 she left to German
to work and failed to take the drugs for several consecutive
days. From November to December 1999 she began to feel
unwell, with asthenia, insomnia, dizziness, and strong he-
addaches, with the sensation of a bigger and full head. She
became progressively more depressed, crying frequently, ha-
vie difficulties in personnel relationships, retiring frequen-
tly to dark rooms. She also noted progressive difficulty in
making simple arithmetic calculus, and frank memory loss.
She looks for us on January 2000. The MRI revealed lesions
of the basal nucleus of the brain, compatible with the clinical
diagnosis of neurological Wilson disease. High signal inten-
sity lesions of the putamina on T2WI were present, sugges-
ting edema and gliosis, as well as slight to moderate cerebral
atrophy (Figs. 1 A, B).
LAB tests revealed: normal hemogram, blood coagulation
and liver function tests, but some abnormalities of copper
biochemistry (Table 1). We immediately initiated therapy
with zinc sulphate 200 mg four times daily, that was main-
tained for one year, returning on that time to a dosage of
200 mg three times daily. Clinically the patient noticed pro-
gressive amelioration during the next three to four months,
and asymptomatic from that point. She comes regularly to
control until January 2001 (Table 1). Serial brain MRI were
performed during the years (July 2000, December 2000, De-
cember 2003, July 2007), which revealed progressive decre-
ase of the radiologic abnormalities, with just some residual
aspects being found (Figs. 2 A, B).
When the patient became pregnant in August 2002, the
same zinc sulphate dosage was maintained. In October 2002
copper biochemistry was stabilized (Table 1). A boy was
born in May 2003, without any perinatal problem; he is now
four-years-old, and has a normal physical and intellectual
development, and normal copper biochemistry. The patient
continued the same treatment and by November 2003 co-
pper biochemistry improved (Table 1). The patient didn’t
visit us, because she lives far from our city, but we know,
by calling her that she maintained the same therapy and felt
well. In September 2004 an interruption of pregnancy was
performed on week 24 due to a prenatal diagnosis of agene-
sis of the corpus callosum associated with ventriculomegal,
confirmed by MRI at 22 weeks of gestation (Figs. 3 A, B). The
amniocentesis realized just before the therapeutic abortion
revealed a normal chromosomal study for the masculine
sex. The necropsy of the foetus showed a complete agene-
sis of the corpus callosum and also a tetralogy of Fallot. No
noxius drug was mentioned during pregnancy. Genealogic
study of both father and mother families were negative for
any suspected genetic disease related to the anomalies de-
tected in the foetus. The patient was advised to take precon-
cepcional folic acid as she decided to get pregnant keeping
the same dosage of zinc sulphate. However, at April 2007
and due to a prenatal ultrasound diagnosis of brain malfor-
mation, a complete agenesis of the corpus callosum associ-
ated with ventriculomegaly (Fig. 4), confirmed by MRI, another
pregnancy interruption was done at 22 weeks of gestation.
Again, no noxius drugs were used during pregnancy. Ne-
cropsy of the foetus revealed complete agenesis of the cor-
pus callosum and also hydroureter and hydronephrosis of
the right kidney. On June 2007 and May 2008 hemogram
and blood coagulation as well as liver function tests were
normal, but copper biochemistry was again slightly lifted.
up (adjusted copper concentration for caeruloplasmin and copper:caeruloplasmin ratio) (Table 1). For that reason we decided to advise the patient to take a zinc sulphate dosage of 200 mg four times daily and to ask the patient to follow a strict diet poor in copper (instead of her regular diet rich in copper). As by the 45th day copper biochemistry was again in normal limits, the dosage of 200 mg three times daily was advised once more. The patient, now 38-year-old, wishing to have a new pregnancy decided not to take a lower dosage of zinc therapy. At that time she found that was already pregnant of 11 weeks; in the first prenatal ultrasound the diagnosis of missed abortion was done. At that time the patient did a tubal ligation.

DISCUSSION

Nonspecific imaging findings in the brain of WD patients include: general atrophy; abnormal gray matter of lentiform, caudate and thalamic nuclei; and abnormal infratentorial white matter. Generalized atrophy suggests a more generalized susceptibility of the nervous system to copper toxicity. The specific neuroimaging findings of WD correspond to the neuropathologic spectrum of spongy to cystic degeneration of gray matter nuclei; central pontine myelinolysis; and softening, gliosis, and demyelination of nerve fibers. Common abnormal MRI findings in WD patients, are increased signal intensity lesions on the T2 weighted images (T2WI) that are thought to reflect oedema, necrosis or cystic changes. A characteristic, although infrequent finding, is the “face of giant panda sign”, due to T2 hyperintensity in the mesencephalon that spares the red nucleus and pars reticulate of the substantia nigra.

Wassener-van Hall, et al defines three types of abnormal gray matter signal intensities (SI) on MRI: type 1, high SI on T2W spin echo (SE) images, with any SI on T1WI; type 2, high SI on T1W inversion-recovery images, with any SI on T2W SE images; type 3, low SI on T2W SE images, with normal SI on T1W inversion-recovery images. In the same study, abnormal white matter was depicted with high SI on T2W SE and T2-weighted gradient-echo images and with mostly low SI on T1W inversion-recovery images. High SI in both gray and white matter on T2WI was the most common finding. MRI morphometry of the midbrain may be useful in the differential diagnosis of different neurodegenerative diseases. Through morphometric measures Semnic demonstrated prominent midbrain atrophy in patients with WD. Abnormal high-signal-intensity lesions in the basal ganglia on T1WI can be seen in children with a clinical picture characterized mostly by liver disease and no severe neurologic symptoms. Possibly these abnormalities could be ascribed to biochemical changes associated with functional alterations that may precede the T2WI seen in more advanced forms of neurologic disease. However the cause of isolated hyperintensity in T1WI remains elusive and Kozic suggested that this hyperintensity might be reversible during the course of chronic chelating therapy.

There are only a few reports regarding the fertility and outcome of pregnancy in WD. In the series of Sinha, et al recurrent abortions and stillbirths were common especially in women with untreated WD, but successful pregnancies and normal full-term delivery occurred in women receiving treatment and also in undiagnosed presymptomatic patients. Other authors found a high occurrence of successive unex-
plained abortions and advise the screening for WD in cases of unexplained recurrent abortions when family history demonstrated consanguinity or neurological, or psychiatric, and/or liver disease. The exact cause of these miscarriages is unclear, but it is believed that increased copper deposition in uterus prevents implantation of the fetus, due to free intrauterine copper derived from non-caeruloplasmin bound copper present in excess in these patients.

However, despite plausible toxicity of copper excess, copper is an essential trace element serving as an important catalytic cofactor in redox chemistry for a large number of proteins required for normal cell function. Copper deficiency gives rise to a multitude of symptoms, well illustrated by Menkes’ disease, an X-linked recessive disorder with a mutated ATP7A gene.

There is increasing evidence that the origin of a significant number of developmental defects may be because of suboptimal nutrition during embryonic and foetal development. Given the multiple roles that copper plays during foetal and embryonic development, the teratogenesis of copper deficiency likely involves an impairment of more than one pathway or process. The structural and biochemical defects...
associated with developmental copper deficiency must arise from several biochemical lesions, the significance of which being related to their temporal expression and severity.

Studies in animals demonstrated that copper deficiency during embryonic and foetal development can result in numerous gross structural and biochemical abnormalities. Changes in free radical defence mechanisms, connective tissue metabolism, and energy production can all contribute to copper deficiency associated dysmorphogenesis. The biochemical alterations hypothetically underlying brain development abnormalities are: (1) reduction in the activity of the cuproenzyme, cytochrome-c oxidase; (2) excessive cellular oxidative damage through reductions in the activity of the oxidant defence enzyme-copper-zinc super oxide dismutase, resulting in peroxidation of lipids and oxidative damage to proteins and nucleic acids; (3) marked reduction of the brain cuproenzyme peptidylglycine monoxygenase, resulting in long-term behavioural and metabolic consequences in view of its impotence to hormone activation; (4) copper and low-molecular-weight copper complexes have angiogenic properties, being reasonable to speculate that altered angiogenesis may contribute to the brain dysmorphology associated with developmental copper deficiency; (6) markedly reduced protein-lysine 6-oxidase activity compromising vessel integrity, secondary to impaired collagen and elastin cross-linking and leading to haemorrhage and blood pooling in the embryonic fetal brain.

The extent to which copper deficiency influences human prenatal development is not known. Buamah, et al demonstrated that low serum copper in non WD pregnant woman during midgestation was associated with an increased risk of anencephaly, and Morton, et al also demonstrated a correlation between low copper in drinking water and the occurrence of neural tube defects. Fosmire refer to the possibility of zinc overt toxicity symptoms with extremely high zinc intakes. At pharmacological intakes of zinc, copper deficiency is also induced and side effects can occur (anemia and leukopenia, abnormalities of immune response and of blood lipid profiles). Nevertheless zinc therapy is considered essential for the treatment of WD.

Consumption of high concentrations of zinc in the diet in experimental animals can induce a secondary copper deficiency as a consequence of decreased copper absorption and utilization. Disease and drug-induced alterations in copper metabolism leading to copper deficiency may result in congenital malformations in experimental animals if maternal plasma copper level is low during the period of organogenesis. Studies in embryo culture systems in animals showed that copper-deficient embryo cultures in copper-deficient serum displayed numerous abnormalities including swollen hindbrain, blisters, blood pooling and distension of the large vessels. It is possible although not proved that copper deficiency at the conception has a causal effect on embryo malformations. It would be important to know if whether the effects of copper deficiency on the foetus or on the embryo are due directly to a deficiency of...
copper in foetal or embryonic cells, or whether they occur in part through indirect effects of copper deficiency on the metabolism of the mother.

However at the level of clinical practice most authors consider that zinc therapy is essentially non toxic. Brewer, et al. report 26 pregnancies of patients treated with zinc acetate resulting in 24 normal infants, 1 with a heart defect, and 1 microcephalic, concluding that zinc is the optimal choice for the pregnancies in women with WD. Notwithstanding the authors refer to the possibility that whatever the drugs utilized in mothers, the major risk of teratogenicity in WD pregnancies is overtreatment, reducing the copper level too low in the mother, and thereby affecting the foetus. However the risk appears to be greater with chelators due to the “pulsatile” nature of this therapy, in contrast with zinc that causes a gradual depletion of copper stores, avoiding blunt “peaks” of blood copper after mealtime. This “levelling” of blood copper levels, as opposed to peaks and through might be better physiologically for the fetus, and offer at least a theoretical advantage to zinc therapy. Due to the above considerations Brewer, et al. reasons that physicians should perhaps aim for reasonable but not tight control of the mother’s copper status during pregnancy. The authors advise to reduce the daily dosage of zinc acetate to half the dosage. We have no experience to advise the same attitude in treating these patients with zinc sulphate during pregnancy.

In this case the agenesis of the corpus callosum and the ventriculomegaly, occurred in association with congenital heart disease and with hydronephrosis. The corpus callosum starts its development at around 9 weeks, and at 20 weeks is normally fully developed. Its maldevelopment may be associated with other brain abnormalities like migrational disorders. The occurrence of the same clinical phenotype may be caused by an extrinsic mechanism, like a teratogenic mechanism or by a gene recessive autosomic or X-linked. The prenatal ultrasound and the necropsy didn’t diagnose any other malformations, namely skeletal or facial. However the extension to which maternal dietary or drug-induced copper deficiency influences human pregnancy is not well understood.

In conclusion, we demonstrated that zinc therapy of neurological Wilson’s disease is clinically and radiologically effective, as was already proved by other authors, if the patients regularly comply with the advised therapeutic scheme. Pregnancy is associated with extraordinary metabolic demands on the mother and the developing foetus. Adequate maternal nutrition is essential for normal embryogenesis. In animals copper deficiency produces effects encompassing intruterine growth retardation and teratogenic effect or embryonic death. We hypothesize that zinc-induced copper deficiency can be a mechanism for embryonic malformations. Eventually a lower dosage of zinc therapy, with strict control of the clinical evolution of the pregnant mother and of the laboratory tests related to copper metabolism, could be the most appropriate attitude in these patients.

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REFERENCES

13. Sturniolo GC, Mestriner C, Irato P, et al. Zinc therapy increases duodenal concentration of metallothionein and iron in
47. Morton MS, Elwood PC, Alberney M. Trace elements in water and congenital malformations of the central nervous system.