Synthesis and use of copper histidinate in children with Menkes disease in Mexico

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Abstract

Menkes disease (MD) is a neurodegenerative and lethal pathology caused by gene mutations of the copper-transporting ATP-7A enzyme; it manifests itself by neurological symptoms and connective tissue changes of varying severity. Timely subcutaneous use of copper histidinate (Cu-His) is determinant for quality of life. We report the first experiences in Mexico on Cu-His synthesis and its safe use in 3 cases where hypocupremia and hypoceruloplasminemia were corroborated. With advice of the Hospital for Sick Children of Toronto Canada, we prepared a 500 µg/mL solution. In all three cases were 250 µg of Cu-His applied without relevant undesirable effects for 30 days. Serum copper (Cu, expressed in µg/L) and ceruloplasmin (Cp, in mg/dL) were determined: case 1, Cu days 0 and 30, 8 and 504 µg/L; Cp days 0 and 30, 4 and 10.75 mg/dL; case 2, Cu days 0 and 30, < 50 and 502 µg/L; Cp days 0 and 30, 2 and 15 mg/dL; case 3, Cu days 0 and 30, 3 and 84.2 µg/L; Cp days 0 and 30, 4 and 10.7 mg/dL. In Mexico, it is possible to safely synthesize Cu-His and treat MD, which must be intentionally sought.

KEY WORDS: Menkes. Histidinate. Copper. Children.

Introduction

Menkes disease, OMIM code # 309400, is a X-linked recessive disorder that is rare (1 in 35000 to 250000 live births), but early lethal and neurodegenerative.^{1,2} It is caused by intragenic mutations or deletions in the gene coding for the ATP7A copper transporter enzyme, which affect multiple enzyme systems (Table 1)^{3,4} and manifest themselves in a clinical spectrum that varies between the severe form (most common) or classic Menkes and occipital horn syndrome (OHS), with manifestations such as severe psychomotor retardation, epileptic encephalopathy, emaciation, failure to thrive, integumentary hypopigmentation, trichodystrophy (pili torti), urocytostrophy, and osteoporosis, with survival of severe cases generally not beyond the third year of life.^{3,5} For treatment and palliation, copper

histidinate (Cu-His) is used as an intervention with potentially significant clinical impact on the central nervous system and quality of life, to the point that in patients who received it early it has prolonged survival to more than 30 years of life in good clinical conditions.⁶⁻⁸ We report the first experiences in Mexico in the synthesis of Cu-His and the findings with its use in three cases.

Method

All reagents were produced by Sigma-Aldrich®. The Cu-His synthesis was carried out following the Hospital for Sick Children of Toronto Canada instructions (August 2013 version, Department of Pharmacy-Compounding). The copper-derived final product expires 56 days after of being synthesized.

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Table 1. Copper-dependent enzymes in mammals and their probable relationship with Menkes disease

Enzyme	Biological activity	Symptom
Cytochrome C oxidase	Cellular respiration	Central nervous system degeneration, ataxia, muscle weakness, respiratory failure
Superoxide dismutase	Free radical sweeping	Central nervous system degeneration
Ceruloplasmin	Iron and copper carrier	Anemia
Hephaestin	Iron transport	Anemia
Tyrosinase	Formation of integumentary pigments	Hypomelanosis
Dopamine beta OH-ase	Catecholamine production	Ataxia, hypothermia, hypotension, diarrhea
Alpha-amidating peptidyl	Peptide hormones activation	Unspecific
Lysyl oxidase	Collagen and elastin cross-links	Premature membrane rupture, cephalohematoma, abnormal facies, high-arched palate, emphysema, hernias, urinary bladder diverticula, arterial aneurisms, joint and skin hyper-laxity, osteoporosis, petechiae, dehiscent wounds, central nervous system degeneration
Sulfhydryl oxidase	Keratin crosslinking	Abnormal hair and dry skin

Source: references 3 and 4.

The Cu-His magistral preparation (500 µg/mL) was carried out as follows:

- Preparation of 0.2N sodium hydroxide solution in 0.9 % sodium chloride solution for injection. Wearing the appropriate protective equipment (safety glasses and gloves), 400 mg of sodium hydroxide were weighed on the analytical balance. Since the substance is supplied as pellets, an adjustment calculation was necessary (e.g. 440 mg q.s. 56 mL). The pellets were placed in a sterile beaker under the laminar flow hood. 40 mL of 0.9 % sodium chloride solution for injection were added to dissolve; the preparation was then stirred. Then, q.s 50 mL, with 0.9 % sodium chloride solution for injection was added in a volumetric flask. The milliliters additional to 50 mL are prepared separately using a syringe and are incorporated to the beaker, where they are mixed.
- Preparation of the Cu-His solution. Wearing appropriate protective equipment (safety glasses and gloves), copper chloride and L-histidine were weighed on the analytical balance. The flask was weighed and quickly returned to the desiccator (copper chloride is hygroscopic). Both powders were placed in the beaker and dissolved in approximately 185 mL 0.9 % sodium chloride solution for injection; the preparation was gently stirred only to dissolve it (the product is sensitive to oxygen). After standardizing, the electrode (between pH 4 and 7) was submerged several times in 99 % alcohol to clean it; then it was carefully

rinsed. The solution was adjusted to a pH of 7.38 to 7.40, using 0.2N of the sodium hydroxide solution; the pH of the solution was determined. Approximately 14 mL plus 10 to 13 drops were taken (depending on NaOH potency more or less were used); after each addition they were mixed well. pH was continuously monitored. Once close to the indicated pH (6.9 to 7.0), half a drop was added and slowly stirred. Upon reaching the indicated pH, the electrodes were cleaned with sterile water and the solution was transferred to a volumetric flask. The volume was adjusted to 200 mL with 0.9 % sodium chloride solution for injection. The pH was measured again to ensure it remained within range (sometimes, extra NaOH drops may be needed). The solution was transferred to a beaker. Copper-histidine solution was drawn in a 20 mL syringe and 3 mL of the solution were filtered using a 0.22 micron disc filter into either 5 or 10-mL vials.

It is important to consider some technical specifications:

- The flasks and beakers used in the preparation of the copper-histidine solution should be covered with aluminum because the product is highly sensitive to light. The hood light should be off during preparation. If copper chloride turns blue/green, it is essential to check with the pharmacist prior to its use.
- Once the first vial is prepared, it must be sent to have the copper level measured.

Figure 1. Cu-His subcutaneous administration quadrants (right and left arm proximal third posterolateral aspect). Source: Reference 8 image recreation.

- The product should remain aqua blue in color. If it is darkened to blue-greenish it means that it is degrading.
- Once finished, the product should not be subjected to impact or shaken.

To avoid degeneration by photic or thermal exposure, 2 mL falcon-type vials with amber color were used and transported on a support base to keep them vertical in containers, whose internal temperature should range from 4 to 10 °C

For its unit dispensation it is suggested to load within the area of pharmaceutical services within minutes prior to its administration, in a room with dimmed light and regulated temperature and a laminar flow hood in a 1 mL syringe with a 27G × 13 mm needle, checking for appropriate coloration and protecting the injection device as soon as possible with dark plastic.

For administration, the deltoid region of both arms was used, divided into nine quadrants to order daily application (Figure 1).⁷ The injection must be administered by the subcutaneous route. The recommended standard dose is 250 µg/dose (0.5 mL) every 24 hours.^{2,3,4}

Results

The solution resulting from the preparation—clear, aqua blue, photosensitive and thermosensitive (Figure 2) was applied without significant undesirable effects related to the site of subcutaneous (deltoid) administration, or relevant systemic effects during patient follow-up. During the first 30 days, daily subcutaneous administration (after informed consent is obtained) of Cu-His 250 µg in patients with Menkes disease generates

Figure 2. Solution appearance. Source: Picture taken at Universidad Autónoma de Nuevo León Faculty of Chemistry laboratory of materials with the first batch of synthesized vials.

changes in serum copper determinations (Cu, reference level 700 to 1750 µg/L) and ceruloplasmin (CP, reference level 22 to 58 mg/dL) (Figures 3 and 4).

Case 1 was detected at 12 months of age, case 2 at 36 months of age and case 3 at four months of age. In all cases, attenuation in the number and duration of infantile spasms was observed, as well as improvement in reactivity and sleep-alertness alternation. Only case 3 had integumentary changes, even with Cu and CP levels lower than reference levels. At the time of this report, spectroscopic measurement and β-microglobulin behavior monitoring was ongoing as follow-up of the tubular function.

Discussion

Cu-His is an endogenous or synthetic molecule that reinforces intracellular uptake of copper and at the same time is a modulator or buffer of copper exchange between the cell and albumin, which regulates its bioavailability.^{2,9}

In Menkes disease, Cu and CP serum levels are negligible, primarily due to an impairment of intestinal absorptive capacity related to ATP7A dysfunction, with supplementary treatment therefore being focused on replacing this element. Once cupremia is recovered, ceruloplasminemia restitution is induced, which assures its efficient delivery to the cells. However, once in the plasmatic compartment, copper high affinity for albumin must be evaded, which reduces its bioavailability. This is where the value of multitasking of molecules such as Cu-His lies, since other molecules such as copper chloride, copper sulfate, copper ethylenediaminetetraacetic

Figure 3. Cupremia after 30 days of Cu-His use in all three cases.

Figure 4. Ceruloplasminemia after 30 days of Cu-His use in all three cases.

acid and cupric albumin have failed to show sufficient clinical impact in comparison with Cu-His.⁹⁻¹²

The recommendation is to use Cu-His in order to improve Cu bioavailability in copper-dependent enzymatic systems through the subcutaneous route, evading the enteral barrier defect and facilitating and capturing copper transfer from copper albumin to improve its bioavailability. This has been established according to several cases of early detection in the range from zero to seven months of age, where neurological evolution prognosis drastically changes, especially if

Cu-His is administered within the first two months of life.^{3,6,9-13} Our cases were 12 and 36 and 4 months of age, with classic Menkes disease evolution and their main problem was nutritional status and refractory epilepsy. There is sparse information available on Cu-His late initiation, which cannot be contrasted with early-initiation chronic use in most reported series (during the first three years of life).^{7,13}

The decision to develop the Cu-His synthesis in Mexico – which had not been possible due to the instability of this substance in an aqueous state and

because the logistical network to allow its safe availability had not been established – was motivated by the possibility of drastically modifying the natural history of future cases and of having a potentially beneficial late intervention, although of uncertain effect, which requires close monitoring.

In counting the obstacles, performing atomic absorption tests and sterility are the procedural steps that require more resources and precision, despite of which they were optimally performed.

In spite of Cu-His late initiation, plasma and clinical improvement changes were observed, and its use will therefore continue with the monitoring of findings and their comparison with those of other series.

We believe that in Mexico it is possible to collaboratively and safely synthesize Cu-His and early accessing to Menkes disease treatment, which involves a deliberate and systematic search of cases throughout the national system, since many subjects die due of the lack of timely detection.

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