Diagnosis and therapy of Menkes syndrome, a genetic form of copper deficiency\textsuperscript{1,2}

Stephen G Kaler

ABSTRACT In the 25 y since copper deficiency was first delineated in persons with Menkes syndrome, advances in our understanding of the clinical, biochemical, and molecular aspects of this rare disorder have surpassed progress in the design of effective therapies. In contrast with purely nutritional copper deficiency, in which copper replacement can be curative, the nature of the basic defect in Menkes syndrome suggests that corrective efforts are likely to be more complicated, a point supported by the cumulative literature on this topic as well as by emerging molecular data. In this paper, certain clinical, biochemical, and molecular aspects of copper histidine treatment in 25 Menkes syndrome patients at the National Institutes of Health are reviewed. The delineation of a distinctive neurochemical pattern in plasma and cerebrospinal fluid, reflecting deficiency of the copper enzyme dopamine \( \beta \)-monooxygenase, is arguably the most important finding in the study of Menkes syndrome. This abnormal pattern has proven extremely reliable as a rapid diagnostic test, enabling early identification of affected infants—a fundamental requirement for improving clinical outcomes. Of 11 patients identified by prenatal or prompt postnatal testing and treated within the first 10 d of age, one walked at 14 mo of age and has normal neurodevelopment at age 3 y and another infant's early progress appears promising. However, five patients died in infancy and neurodevelopmental outcome was suboptimal in four others. Consideration of additional therapeutic strategies seems necessary, therefore, for most patients and families facing this troublesome form of copper deficiency. Am J Clin Nutr 1998(suppl):67:1029S–34S.

KEY WORDS Menkes syndrome, copper, dopamine \( \beta \)-monooxygenase, catecholamines, mutations, splicing, infants

INTRODUCTION

Menkes syndrome is an example of a naturally occurring human copper deficiency, resulting from defects in an X chromosomal gene that normally encodes a copper-transporting ATPase (1–3). Because of reduced or absent amounts of this gene product, or because of alterations that impair the molecule's copper transport function, Menkes syndrome patients fail to absorb copper from the gastrointestinal tract in quantities adequate for meeting nutritional needs (4, 5). These needs seem particularly acute during the initial 12 mo of life, when the velocity of brain growth and motor neurodevelopment (processes that require copper) is normally most rapid (6). Consequently, although 8–10 wk of good health after birth is typical in Menkes syndrome, the untreated condition is inevitably associated with significant neurologic abnormalities; death usually occurs during infancy or early childhood (7).

HISTORY OF MENKES SYNDROME

As early as the 1930s, veterinary scientists in Australia described the important role of copper in mammalian neurevelopment through the association of copper deficiency with demyelinating disease in ataxic lambs (8). The animals' mothers had grazed in copper-deficient pastures throughout pregnancy, and the offspring suffered cerebral demyelination and gross pathologic changes including porencephaly. Some 40 y later, Danks (4, 5, 9), a physician, identified Menkes syndrome as a human example of abnormal neurodevelopment due to copper deficiency. Danks's discovery was based on his recognition that the unusual hair of infants with Menkes syndrome appeared similar in texture to the brittle wool of sheep raised on copper-deficient soil in Australia. He measured serum copper in seven Menkes syndrome patients and found low concentrations in all cases. Serum concentrations of ceruloplasmin, an important copper enzyme, were also subnormal. Thus, observations four decades apart in Australia concerning the effects of copper deficiency became extremely relevant to a human inborn error of metabolism.

This important biochemical finding sparked renewed interest in the clinical phenotype delineated carefully 10 y earlier by Menkes and his colleagues (10) at Columbia University in New York. Menkes described five male infants in a family of English-Irish heritage affected with a distinctive syndrome of neurologic degeneration, peculiar hair, and failure to thrive. The infants appeared normal at birth and throughout their first several months of life, but then experienced seizures and developmental regression and ultimately died between the ages of 7 mo and

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3.5 y. The pedigree of the family strongly suggested that the condition was an X-linked genetic disease. Subsequent case reports confirmed that kinky-hair disease was a newly recognized syndrome with unique clinicopathologic features (11–18). During the next 20 y, additional published descriptions of Menkes syndrome patients brought attention to the clinical, biochemical, and pathologic spectrum of the disorder. Reports concerning treatment with copper supplements (compiled in reference 7, Table 4) were generally disheartening, with some exceptions.

In 1993 identification of the Menkes gene by positional cloning was reported by groups from San Francisco; Melbourne and Ann Arbor, MI; and Oxford, United Kingdom, and Copenhagen (1–3). These landmark discoveries disclosed that the Menkes gene product is a member of a highly conserved family of cation-transporting ATPases, molecules that function in the transport of ions across cellular and intracellular membranes (19, 20). In conjunction with previous data characterizing the biochemical abnormalities in Menkes syndrome patients and their cultured cells (21–27), the findings suggested that the basic defect in Menkes syndrome involves a failure of copper exit from cells. As a result of the gene’s identification and the insights into mammalian copper metabolism that this discovery provided, Menkes syndrome is the subject of renewed scientific scrutiny today. Thus, 35 y after the initial clinical description, the history of this interesting disorder continues to unfold.

DIAGNOSIS OF MENKES SYNDROME: THE CLINICAL PHENOTYPE

Menkes syndrome is a relatively rare condition with incidence estimates ranging from 1 in 100,000 live births to 1 in 250,000 (7). On the basis of recent annual birth rates in the United States (=3.5 million), 15 to 35 affected babies would be expected to be born in this country each year. One-third of these cases are predicted to be nonfamilial, representing new mutations (28).

As an X-linked disease, Menkes syndrome typically occurs in males who at 2–3 mo of age show loss of previously obtained developmental milestones and the onset of hypotonia, seizures, and failure to thrive. Characteristic physical changes of the hair and facies, in conjunction with typical neurologic findings, often suggest the diagnosis. The signs and symptoms of 127 patients reported in the medical literature up to 1985 have been compiled (29). The less distinctive appearance of very young affected infants before the onset of neurodegeneration is discussed separately below. In the natural history of classic Menkes syndrome, death often occurs by 3 y of age, although normal tracings have been recorded in some classically affected individuals (32). Brain magnetic resonance imaging findings typically include white matter abnormalities reflecting impaired myelination, tortuosity of cerebral blood vessels, or diffuse atrophy with ventriculomegaly (33–35). Cystography or pelvic ultrasound reveals diverticula of the urinary bladder in nearly every patient (36, 37). Radiographs often disclose abnormalities of bone formation in the skull (wormian bones), long bones (metaphyseal spurring), and ribs (anterior flaring and multiple fractures) (38–41).

DIAGNOSIS OF MENKES SYNDROME: THE BIOCHEMICAL PHENOTYPE

The biochemical phenotype in Menkes syndrome involves low concentrations of copper in plasma, liver, and brain because of impaired intestinal absorption (5), reduced activities of numerous copper-dependent enzymes (42–44), and paradoxical accumulation of copper in certain tissues (duodenum, kidney, spleen, pancreas, skeletal muscle, and placenta) (45–47). The copper retention phenotype is also evident in cultured fibroblasts and lymphoblasts, in which reduced egress of radiolabeled copper can be shown in pulse-chase experiments (23–27, 48). This constellation of biochemical findings denotes a primary defect in copper transport that begins with impaired absorption at the intestinal level and continues with failed utilization and handling of whatever copper is conveyed to other cells in the body. The biochemical features important for diagnosis are summarized in Table 1.

Certain features of Menkes syndrome can be clearly related to deficient activity of specific copper-requiring enzymes and one can speculate on the effects that reduced activity of other copper enzymes would produce. Partial deficiency of dopamine b-hydroxylase, or DBH), a critical enzyme in the catecholamine biosynthetic pathway, is responsible for a distinctively abnormal plasma and cerebrospinal fluid neurochemical pattern in Menkes syndrome patients (49, 50).

In our experience, the ratio of a proximal compound in the pathway, dihydroxyphenylalanine (also called DOPA), to a distal metabolite, dihydroxyphenylglycol, provides a better index of DBH deficiency in Menkes syndrome patients than norepinephrine concentrations alone. Plasma and (especially) cerebrospinal fluid concentrations of norepinephrine, the direct product of DBH, are relatively well maintained in some Menkes syndrome patients, presumably because of suitable compensatory mecha-
TABLE 1
Biochemical diagnosis of Menkes syndrome

<table>
<thead>
<tr>
<th>Local hospital or clinic</th>
<th>Serum copper &lt; 11 μmol/L (&lt; 70 μg/dL)1</th>
<th>Serum ceruloplasmin &lt; 200 mg/L2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pili torti on microscopic examination of hair</td>
<td></td>
</tr>
<tr>
<td>Specialized testing</td>
<td>Copper egress in cultured fibroblasts</td>
<td>Plasma catecholamine analysis2</td>
</tr>
<tr>
<td></td>
<td>Placental copper concentration2</td>
<td>Direct mutation analysis</td>
</tr>
</tbody>
</table>

1 Unreliable in newborns during the first several weeks of life.
2 Rapid diagnostic test in newborns.

nisms (50). Interestingly, recent animal studies of copper deficiency indicate that norepinephrine concentrations are maintained at near normal levels in certain regions of the brain (eg, hypothalamus) (51). Clinical features of Menkes syndrome patients potentially attributable to partial DBH deficiency include temperature instability, hypoglycemia, and eyelid ptosis, autonomic abnormalities that may result from selective loss of sympathetic adrenergic function. Similar clinical problems have been described in patients with a congenital absence of DBH (52, 53).

A recently described copper-dependent enzyme, peptidylglycine monooxygenase, is required for removal of the carboxy terminal glycine residue characteristic of numerous neuroendocrine peptide precursors (eg, gastrin, cholecystokinin, vasoactive intestinal peptide, corticotropin-releasing factor, thyrotropin-releasing hormone, calcitonin, and vasopressin) (54, 55). Failure to amidate these precursors can result in 100- to 1000-fold diminution of bioactivity compared with activity of the mature, amidated forms. Recent studies documented some decline in peptidylglycine monooxygenase activity in rat brains during copper deficiency (56). Partial peptidylglycine monooxygenase deficiency could be expected to produce a wide range of physiologic effects and may well contribute to the Menkes syndrome phenotype.

Deficient cytochrome-c oxidase activity is probably a major factor in the neuropathology of Menkes syndrome. Effects on the brain are similar to those in persons with Leigh disease (subacute necrotizing encephalomyelopathy) in whom cytochrome-c oxidase deficiency is caused by complex IV respiratory chain defects (57, 58). As in Leigh disease patients, Menkes syndrome patients do not have the severe lactic acidemia that is associated with other complex IV defects (59). Cytochrome-c oxidase deficiency probably also contributes peripherally to the hypotonia and muscle weakness evident in Menkes syndrome patients.

Reduced activity of protein-lysine β-oxidase (also called lysyl oxidase), another copper enzyme, has major clinical consequences in Menkes syndrome. This enzyme normally acts to deaminate lysine and hydroxylsine as the first step in collagen cross-link formation (60). Decreased lysyl oxidase activity significantly reduces the strength of connective tissue in numerous organs and tissues. In Menkes syndrome patients, vascular tortuosity (22), bladder diverticula (36, 37), and gastric polyps (61, 62) are all thought to result from lysyl oxidase deficiency. Of the cuproenzymes, lysyl oxidase appears to be among the most sensitive to defects in the Menkes copper ATPase (63, 64), although the precise reasons for this remain to be fully clarified.

Deficiency of Cu/Zn superoxide dismutase (SOD) in Menkes syndrome (42) could lower protection against oxygen free radicals and theoretically have cytotoxic effects. Localized brain damage due to such oxidant stress has been postulated as the pathogenic basis of Parkinson disease (65), and mutations in the Cu/Zn SOD gene have been associated with amyotrophic lateral sclerosis, a motor neuron disease of adult onset (66). In a recent postmortem immunohistochemical study of brains from subjects with Menkes syndrome (67), increased manganese SOD immunoreactivity was shown in addition to decreased Cu/Zn SOD reactivity, suggesting that a compensatory protective mechanism against superoxide toxicity was active in these tissues. In addition, mice with a complete absence of Cu/Zn SOD show normal neurodevelopment (68). In light of these combined data, the relative contribution of partial SOD deficiency to the neurodegeneration of Menkes syndrome remains difficult to assign.

DIAGNOSIS OF MENKES SYNDROME: THE NEONATAL PERIOD

Classic Menkes syndrome often escapes attention during the newborn period because of its subtle manifestations in neonates (69). However, several nonspecific physical and metabolic findings are commonly cited when birth histories of these babies are reviewed. These include premature labor and delivery, large cephalohematomas in cases in which vaginal birth occurred, hypothermia that necessitated warming lights or an isollette, hypoglycemia for which early feeding or support with intravenous glucose was instituted, and jaundice that required several days of phototherapy. Pectus excavatum and inguinal or umbilical hernias are found at birth in some affected patients.

Occasionally, unusual hair pigmentation may suggest the diagnosis in newborns. Often, however, the neonatal appearance of the hair is unremarkable. As in healthy babies, Menkes syndrome newborns may have no hair or have normally pigmented hair. The pili torti found on microscopic examination of hair from older Menkes syndrome patients is usually not evident in the hair of affected newborns. Neurologically, newborns with Menkes syndrome generally appear healthy.

In addition to the subtle clinical manifestations in affected neonates, early diagnosis is complicated by the unreliability of the usual biochemical markers—low serum copper and ceruloplasmin concentrations—during the first several weeks of life. Copper and ceruloplasmin concentrations are low in healthy newborns (70, 71) and overlap concentrations found in Menkes syndrome patients. A definitive diagnostic test, copper egress in cultured fibroblasts, requires propagation of cells obtained from a skin biopsy for at least several weeks before the assay is performed. Because optimal success of any therapeutic strategy for this condition requires recognition of affected patients before the onset of neurologic symptoms, rapid tests that can reliably diagnose or exclude Menkes syndrome during the neonatal period are critical. One such test recognized recently is plasma catecholamine analysis: distinctively abnormal plasma catechol concentrations, indicating deficiency of dopamine β-monooxygenase, have been shown in affected newborns (49, 50) as well as in a fetus with Menkes syndrome (72). Preliminary data also suggest that plasma obtained at birth from umbilical cord blood reflects the abnormal pattern (73). We currently consider the plasma catechol profile the most rapid and reliable marker for Menkes syndrome in the early neonatal period, during which other tests are ambiguous. Placental copper concentration (increased in Menkes syndrome) represents another reliable biochemical marker for neonatal diagnosis (47, 74).
TREATMENT OF MENKES SYNDROME

At the National Institutes of Health, 25 patients with Menkes syndrome have been treated with copper histidine. Of 11 infants aged <1 mo, 5 died despite very early treatment; of 11 infants in whom treatment was started at older ages, 6 died; and of 3 patients with milder phenotypes, all survived. This experience is similar to that described in the cumulative literature on copper replacement therapy (tabulated in reference 7). Certain patients who are treated from an early age show outcomes better than expected, and some even attain normal neurodevelopmental milestones. However, other patients do not fare well despite early medical intervention. When treatment is commenced at an older age after the onset of symptoms, significant neurologic recovery is not possible, although reduced irritability, calmer sleeping, and slight advances in infant personal and social development are sometimes reported by parents as small joys in the face of the disease. A formal rebuttal of a criticism concerning the model of Menkes syndrome, ie, normal mRNA quantity (81) and residual normal splicing.

In my opinion, several issues must be addressed in configuring improved therapeutic strategies for Menkes syndrome: 1) the block in intestinal absorption of copper must be bypassed, 2) affected infants must be identified and treatment started early in life, 3) circulating copper must be delivered to the brain, and 4) copper must be available within cells to enzymes that require it as a cofactor. Parenteral administration of copper fulfills the first requirement and progress has been made in presymptomatic diagnosis, as noted above. However, issues 3 and 4 represent significant obstacles in many affected patients and underscore the differences between this condition and nutritional copper deficiency.

We showed recently that a homologue of the Menkes gene is expressed in astrocytes from rat brain (76), confirming an earlier biochemical suggestion (77) that this glial cell type is involved in copper delivery to the brain. In astrocytes, neurons, and intestinal mucosal cells, the Menkes ATPase presumably acts to make copper available to secreted cuproenzymes and to direct copper exit. Treatment by copper injections, which bypasses only the initial metabolic block (gastrointestinal absorption into the bloodstream), would not be expected to meaningfully improve outcomes in patients whose mutations entirely eliminate normal copper transport beyond that initial block. Conversely, one could predict that early copper replacement might be most effective in patients whose mutations allow some residual transport activity. Although such treatment-responsive defects would still be severe enough at the gastrointestinal level to impair delivery of normal amounts of copper into the bloodstream, if the blood copper concentration was normalized via daily injections, copper-binding sites of partially functional molecules in various cells could be saturated and some copper transport could proceed. In the brain itself, residual functional MNK ATPase could increase delivery of copper across cerebral capillary endothelial cells and astrocytes that make up the blood-brain barrier, and thus make copper available to central nervous system neurons for incorporation into cuproenzymes such as cytochrome-c oxidase.

Evidence in support of this hypothesis can be drawn from 1) clinical outcomes in several treated patients whose mutations have been determined and characterized (78–80); 2) analyses of the molecular defect in the treatment-responsive brindled mouse model of Menkes syndrome, ie, normal mRNA quantity (81) and a small in-frame deletion (82); 3) older case reports in the literature citing the mixed success of early copper replacement therapy (83–88); and 4) molecular findings in patients with milder Menkes syndrome phenotypes, including occipital horn syndrome (64, 89). These latter individuals, who did not receive early copper replacement, showed relatively less severe neurologic symptoms, presumably related to a considerable amount of residual copper transport; 20–35% of normal Menkes messenger RNA was present in cultured cells from two of these patients (89). In comparison, patients with the classic disease who respond to early treatment may have considerably less residual ATPase, but with copper repletion, the kinetics of transport could be enhanced and lead to improved neurodevelopmental outcomes. Examples of potentially copper-responsive defects include small in-frame deletions and insertions and certain missense mutations, as well as splice junction mutations with some residual normal splicing.

The relative importance of mutation type and other factors (eg, genetic background) in the variable response to early copper treatment needs to be dissected further. It remains possible that even patients with complete deletions of the Menkes gene could respond favorably to early treatment, if alternative pathways were available for copper delivery to the brain and transport within neurons. It appears clear, however, that early treatment with copper histidine is not uniformly successful in Menkes syndrome patients. Although this treatment should still be offered to affected infants identified at an early age, other therapies also need to be considered. Improved understanding of the copper transport process mediated by the Menkes syndrome gene product and its prokaryotic and eukaryotic homologues may eventually facilitate this.

REFERENCES


