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Wilson's disease with cerebral manifestation: monitoring therapy by CSF copper concentration

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Abstract The clinical courses, cerebrospinal fluid (CSF) and serum copper concentrations and urinary copper excretions under different schemes of drug treatment in four patients with cerebral manifestations of Wilson's disease were monitored over 6–11 years. CSF copper concentration measurements were performed from the beginning of therapy onwards in three patients and from 16 months after initial treatment onwards in the fourth. CSF copper levels decreased slowly over the years in parallel with clinical improvements, and increased in one patient who interrupted therapy for 2 years. These findings confirm our hypothesis that the concentration of copper in the CSF is a valuable quantitative parameter reflecting the normalization of copper in the brain. Copper measurements during phases of initial neurological deterioration in two patients receiving D-penicillamine, and in one patient receiving D-penicillamine and zinc sulphate, revealed decreased free serum copper and CSF copper levels.

Key words Wilson's disease
Cerebrospinal fluid · Copper
Diagnosis · Therapy

Introduction

Owing to an autosomal recessive defect on chromosome 13 [12] patients with Wilson's disease accumulate copper in the liver and in other organs, particularly in the central nervous system. Therapy aims at a reduction of tissue copper deposits and at a binding of copper to the protein metallothionein in the liver. Therefore, copper balances should be negative at the start of therapy and later zero.

The most popular drug is the chelating substance D-penicillamine [7, 34, 37, 42, 48], which increases urinary copper excretion and the synthesis of metallothionein in liver cells [3, 33]. In most patients with neurological manifestations of Wilson's disease, symptoms decrease in severity or regress completely after a few years of therapy. However, because of toxic and allergic side-effects, therapy with D-penicillamine has to be stopped in 10–30% of patients [1, 7, 34, 37]. Nevertheless, treatment has to be continued as otherwise rapid and often fatal clinical deterioration occurs within a few years [32].

The chelating agent triethylene tetramine dihydrochloride (trien) has proven to be an effective drug in the therapy of Wilson's disease and is well tolerated [10, 32, 43–46]. But experience with this substance is limited to a few patients. It is not known whether neurological deterioration can occur during the initial months of therapy, as observed in up to 50% of patients treated with D-penicillamine [6, 13, 17, 36].

Some authors have proposed zinc sulphate or zinc acetate as therapeutic alternatives to the initial and mainte-

nance therapy of Wilson's disease [3, 4, 8, 16, 18, 19, 25, 40]. Zinc blocks gastrointestinal copper absorption by inducing mucosal metallothionein synthesis, and also induces hepatic metallothionein synthesis [8, 22, 29]. It produces a modest negative copper balance. Therefore, it is not clear whether zinc monotherapy leads to a sufficiently negative copper balance in the initial treatment phase and/or in patients with severe liver disease [9, 37].

Recently, some patients have been treated with tetrathiomolybdate, which binds copper in the gastrointestinal tract and in serum [9, 24, 47, 49].

Treatment of Wilson's disease should be effective, adjusted to the individual metabolic situation and have no negative side-effects. The therapeutic efficacy of the various drugs can be monitored by the clinical course, liver biopsies [37], the serum copper level and the urinary copper excretion [5], an oral copper absorption test [15, 38, 39, 51], neurophysiological [14, 26, 30], neuroradiological [36, 41, 52] and ophthalmological examinations [23, 35]. But none of these investigations allows a quantitative assessment of the normalization of the copper level in the brain. We observed that the concentrations of copper in the cerebrospinal fluid (CSF) were elevated in patients with cerebral manifestation of Wilson's disease decreasing slowly over the years in parallel with clinical improvements [50]. Therefore we hypothesized that the CSF copper level reflects copper normalization in the central nervous system (CNS). In four of our patients we observed the clinical courses and measured the serum and CSF copper concentrations for 5 further years in order to evaluate the relevance of CSF copper concentration for monitoring therapy.

We also measured CSF and serum copper concentrations during episodes of neurological deterioration during the first months of therapy in three of our patients.

Patients and methods

The clinical courses, serum and CSF copper levels and urinary copper excretions of four patients with cerebral manifestation of Wilson's disease (three female, one male; born between 1957 and 1962) over 6–11 years are reported. CSF copper measurements were performed from the beginning of therapy onwards in three patients, and from 16 months after the initiation of treatment onwards in the fourth.

Medication

Normally patients took D-penicillamine, trien or zinc sulphate 1 h before meals. However, at home patient 1 took the total daily doses of zinc sulphate and D-penicillamine together after dinner, although advised differently. Patient 3 took trien 1 h before meals and zinc sulphate 1 h afterwards.

Sample preparation

CSF was obtained by lumbar puncture and collected in copper-free plastic tubes. Spinal needles (Becton Dickinson, Madrid, Spain) and cannulae (Dispomed Witt OHG, Gelnhausen, Germany) were checked with copper-free water. No copper contamination could be observed. Urine was collected in plastic containers which had been washed about six times with 16% acid until no copper contamination could be detected in the cleaning water.

Copper measurement

Copper was measured by flameless atomic absorption (Perkin Elmer, HGA 500, Überlingen, Germany). The main resonance line was 324.7 nm with deuterium background compensation and argon as a protecting gas; the sample volume was 20 µl using an autosampling system [11]. CSF and urine were measured undiluted. Serum was diluted 1:20, 1:15, 1:10 or less. In all cases the appropriate standards were used with the sample.

Ceruloplasmin measurement

Ceruloplasmin was determined immunoelectrophoretically [21] (LC Paritgen, Behringwerke, Marburg, Germany) until 1989 and nephelometrically (Beckman array; Beckman Instruments, Brea, Calif., USA) after that date.

Case reports

Patient 1 (Table 1)

This female patient, born in 1957, had observed tremor in both hands since November 1985, impaired vision since January 1986 and dysarthria, ataxia and drooling since July 1986. On admission in August 1986 she had a slight organic brain syndrome, generalized rigidity and an intention tremor which increased under emotional stress. CSF copper concentration was elevated. Therapy was initiated with 800 mg/day zinc sulphate. Two weeks later 1200 mg/day D-penicillamine was added. The patient's condition started to improve after 2 weeks, but deteriorated markedly after 10 weeks of therapy. She needed help with dressing and feeding because of the severity of her intention tremors. CSF copper concentration was reduced from 93 to 71 µg/l, while the free serum copper level dropped. Therapy was continued with a reduced dose of 600 mg/day D-penicillamine and 800 mg/day zinc sulphate. A slow but progressive clinical improvement occurred over the following 15 months. Free serum copper was markedly reduced, there was hardly any free serum copper detectable, and urinary copper excretion was low. The patient refused further lumbar punctures. She did not attend follow-up examinations for several years but continued with her medication. She delivered a normal daughter in the 35th week of gestation 3 years after the initiation of therapy. She has been symptom-free since 1989. Neurological examination in October 1992 revealed only slight dysarthria and discrete rigidity in both legs.

Patient 2 (Table 2)

This male patient, born in 1958, was admitted owing to affective lability, headaches and a slight dysarthria. Therapy was initiated with 900 mg/day D-penicillamine in January 1983. About 2 months

Table 1 CSF and serum copper concentrations, serum ceruloplasmin concentrations, urinary copper excretions and therapy of patient 1

	CSF	Serum		Copper excretion	Therapy	Therapy since
	Copper (μg/l)	Copper (μg/l)	Ceruloplasmin (mg/l)	Urine (μg/day)	Oral medication (mg/day)	
Normal ranges[a]	6–35	760–1800	150–600	10–60		
Date						
20.08.86	93	550	68	154	None	26.08.86
05.09.86		390		85	$ZnSO_4$ 800	
12.09.86		292	30	1107	D-penicillamine 1200 + $ZnSO_4$ 800	08.09.86
19.09.86		322		1016	D-penicillamine 1200 + $ZnSO_4$ 800	
15.10.86		215	62	1467	D-penicillamine 1200 + $ZnSO_4$ 800	
17.11.86	71	120	46	539	D-penicillamine 600 + $ZnSO_4$ 800	November 1986
26.01.87		62	25	89	D-penicillamine 600 + $ZnSO_4$ 800	
05.12.87		165	59	49	D-penicillamine 600 + $ZnSO_4$ 800	
30.10.92		380	156		D-penicillamine 600 + $ZnSO_4$ 800	

[a] Reference ranges from healthy subjects [50]

Table 2 CSF and serum copper concentrations, serum ceruloplasmin concentrations, urinary copper excretions and therapy of patient 2

	CSF	Serum		Copper excretion	Therapy	Therapy since
	Copper (μg/l)	Copper (μg/l)	Ceruloplasmin (mg/l)	Urine (μg/day)	Oral medication (mg/day)	
Normal ranges[a]	6–35	760–1800	150–600	10–60		
Date						
08.01.83	81	630	53	348	None	
01.04.83	76	480	73	2421	D-penicillamine 900	January 1983
30.04.83		292	30	2437	D-penicillamine 900	
08.08.83	88	94	23	625	D-penicillamine 900	
20.01.84	65			624	D-penicillamine 1200	
31.08.84	51	75	46	542	D-penicillamine 1200	August 1983
10.05.85	47	123	29	869	D-penicillamine 1200	
26.10.85	39	143		646	D-penicillamine 1200 + $ZnSO_4$ 1200	July 1985
15.07.86	28	131	47	488	D-penicillamine 1200 + $ZnSO_4$ 1200	
07.04.87	29	145	43	316	D-penicillamine 1200 + $ZnSO_4$ 1200	
05.10.87	24	132	52	464	D-penicillamine 1200	September 1985
25.03.88	22	163	63	520	D-penicillamine 1200	
20.07.89	19	336	93	1038	D-penicillamine 1200	
13.04.91	17	402	146	768	D-penicillamine 1200	

[a] Reference ranges from healthy subjects [50]

later his dysarthria, emotional instability, concentration and mnestic functions worsened. Measurements revealed reduced CSF and free serum copper concentrations. Treatment was not changed and clinical improvement started from 3 months after initial therapy. After 7 months, dysarthria was no longer detectable and emotional stabilization was noted, but CSF copper concentration had increased to 88 μg/l compared with 81 μg/l before therapy. Therefore, medication with D-pencillamine was increased to 1200 mg/day. During the further course the CSF copper concentration normalized slowly over 3 years. From July 1985 until September 1987 the patient was administered 800 mg/day zinc sulphate additionally. In October 1985 a slight affective lability was the only clinical abnormality. From 1988 onwards, the results of all clinical examinations were normal.

Table 3 CSF and serum copper concentrations, serum ceruloplasmin concentrations, urinary copper excretions and therapy of patient 3

	CSF	Serum		Copper excretion	Therapy	Therapy since
	Copper (µg/l)	Copper (µg/l)	Ceruloplasmin (mg/l)	Urine (µg/day)	Oral medication (mg/day)	
Normal ranges[a]	6–35	760–1800	150–600	10–60		
Date						
16.10.81		1016	140	708	None	
26.10.81		825		1890	D-penicillamine 900	22.10.81
25.11.81		508	130	2010	D-penicillamine 900	
02.02.82		698		1690	Trien 1800	15.12.81
19.03.82			140	1500	Trien 1500	March 1982
15.04.82				702	Trien 1500	
21.07.82				425	Trien 1500	
08.03.83	92	260	20	351	Trien 1500	
13.10.83	64	130	30	262	Trien 2400	09.03.83
19.04.84	57	236		291	Trien 2400	
10.11.85		376	115	153	Trien 1800 + $ZnSO_4$ 600	March 1985
29.01.86	35	490	138		Trien 1800 + $ZnSO_4$ 600	
18.07.86	18	324	136	225	Trien 1500 + $ZnSO_4$ 600	July 1986
09.02.87	18	304	121	82	Trien 1500 + $ZnSO_4$ 600	
10.07.88		520	170	112	Trien 1500 + $ZnSO_4$ 600	
11.03.90		509		390	Trien 1500 + $ZnSO_4$ 600	
11.05.92		492	124	274	Trien 1500 + $ZnSO_4$ 600	

[a] Reference ranges from healthy subjects [50]

Table 4 CSF and serum copper concentrations, serum ceruloplasmin concentrations, urinary copper excretions and therapy of patient 4

	CSF	Serum		Copper excretion	Therapy	Therapy since
	Copper (µg/l)	Copper (µg/l)	Ceruloplasmin (mg/l)	Urine (µg/day)	Oral medication (mg/day)	
Normal ranges[a]	6–35	760–1800	150–600	10–60		
Date						
19.05.85	60	550	60	176	None	
15.06.85				165	$ZnSO_4$ 600	09.06.85
02.07.85		480		1648	$ZnSO_4$ 600 + D-penicillamine 900	25.06.85
09.09.85	51	312	110	1206	D-penicillamine 900	03.07.85
17.01.86	40	330	85	588	D-penicillamine 900	
11.06.86	40	198	58	295	D-penicillamine 900	
11.02.87	36	290	38	451	D-penicillamine 900	
24.02.88	44	338	72	139	None	March 1987
25.02.89	58	480		47	None	
28.03.90		440		1249	D-penicillamine 900	September 1989
13.09.91	42	315	79	681	D-penicillamine 900	
05.03.92		350	126		D-penicillamine 900	

[a] Reference ranges from healthy subjects [50]

Patient 3 (Table 3)

This female patient, born in 1962, was admitted in 1981 with rigidity, hypomimia, drooling, dysarthria and affective lability, which had developed over 5 months. Therapy with D-penicillamine was started on 22 October 1981. Four weeks later she developed a generalized exanthema and agranulocytosis leading to septicaemia and pneumonia. D-penicillamine was stopped. She was treated at the intensive care unit and worsened neurologically so that she was not able to rise or to eat without help. Two weeks later therapy was continued with trien. In the following weeks she improved slowly and was able to walk and eat without help after 6 weeks. Following a phase of irregular medication and an abortion there was an increase in extrapyramidal symptoms and a psychotic decompensation 6 month later, leading to psychiatric hospitalization for 4 weeks. Her condition stabilized under regular medication and she improved again. Another phase of worsening occurred 1 year after initiation of therapy, but also after irregularities of medication. Copper concentration in the CSF was elevated, at 92 µg/l. Medication with trien was increased to 2400 mg/day. The neurological symptoms improved continuously during the following years. In March 1985, 600 mg/day zinc sulphate was added to the therapy regimen. Trien was reduced to 1800 mg/day in March 1985 and to 1500 mg/day in July 1986. The copper concentration in the CSF normalized slowly over 4 years. Further clinical examination in 1988, 1990 and 1992 revealed only discrete affective lability and a mild extrapyramidal and cerebellar syndrome with discrete rigidity and with intention tremor when under emotional stress.

Patient 4 (Table 4)

This female patient, born in 1957, had observed dysarthria, dysphagia, early fatigability and motor disturbances since the beginning of 1984. On admission in May 1985 she had a marked organic brain syndrome with impaired mnestic functions and concentration, affective lability and a mild extrapyramidal syndrome with hypomimia, bradydiadochokinesia, ocular dysmetria and dysarthria. CSF copper concentration was elevated at 60 µg/l and copper balance was positve. The patient was given zinc sulphate over 2 weeks, followed by zinc sulphate and D-penicillamine over 1 week and D-penicillamine alone during the further course. Two months after initiation of therapy her dysarthria, dysphagia and difficulties in concentration started to worsen and she was readmitted. CSF copper concentration had decreased and the free serum copper concentration was nearly zero. Therapy was continued without change and a slow clinical improvement started about 4 months after the initiation of therapy. This was interrupted by a phase of deterioration 1 year later when she took her tablets irregularly during her divorce. In March 1987 the CSF copper level had reached the upper limit of the normal range and the patient had undergone further clinical improvements. However, she broke off therapy. After 1 year without treatment she had made further clinical improvement and no changes were observed in visual and brain-stem evoked potentials, but the CSF copper level had already increased from 36 µg/l to 44 µg/l. Clinical deterioration was rapid another year later, with a marked increase in rigidity and the organic brain syndrome. At this time CSF copper level had increased further to 58 µg/l and the free serum copper level was elevated again. Therapy was continued with D-penicillamine, but the patient had, in addition, begun to abuse alcohol. At further follow-up examinations the clinical syndrome did not change over months. A slight improvement was noted in March 1992. The CSF copper concentration had fallen again to 42 µg/l.

Discussion

The CSF copper concentration was elevated in all four patients before treatment was initiated (patients 1, 2, 4) or 1 year later (patient 3) and decreased slowly over the years in parallel with clinical improvements. As the CSF coeruloplasmin concentration is 0.8–2.0 mg/l in healthy subjects and even lower in patients with Wilson's disease (values of 0.3–0.45 mg/l in our patients, data not shown) the CSF copper can be regarded as mainly free and non-ceruloplasmin-bound. However, the free serum copper concentration calculated from the total serum copper and the serum ceruloplasmin levels, assuming that ceruloplasmin contains 0.3% copper [3, 31], had already fallen to nearly zero after 6 months of therapy in all four patients. From these data we conclude that the elevated CSF copper concentration cannot only be a result of an elevated free serum copper level but rather reflects the copper concentration in the CNS. Elevated CSF copper levels in patients with cerebral manifestation of Wilson's disease, which decrease slowly over years of therapy, were also reported by Kodama et al. [20].

The course of the copper concentration in patient 4 indicates that in this patient the CSF copper level was more sensitive in the detection of the reaccumulation of copper in the brain compared with clinical and neurophysiological methods. She stopped her medication for 2 years. After 1 year without therapy no changes were noted in her clinical and neurophysiological examinations but the CSF copper level had already increased from 36 µg/l to 44 µg/l. Rapid neurological deterioration was observed about 2 years later. At this time her CSF copper concentration had increased further to 58 µg/l.

An unsolved and serious problem in the therapy of Wilson's disease with neurological manifestation is the neurological deterioration during the first months of D-penicillamine therapy, which is observed in up to 50% of patients [6, 8, 13, 17, 36]. Some patients never return to their status before therapy and in some patients without neurological abnormalities these develop during initial therapy [6, 13, 36]. As a possible explanation of this phenomenon Brewer et al. [6] proposed that hepatic copper might be mobilized during the initial treatment, causing an increase of serum copper so that copper storage in the CNS continues, but they did not confirm this hypothesis by clinical and laboratory data. Pall et al. [27, 28] argued that an alternative explanation would be that copper is moved from protein complexes in nerve cells and acts toxically to biomolecules such as in peroxidation of membrane lipids [2].

All our four patients had neurological deteriorations during their first treatment phases, but in patient 3 the first deterioration was during an interruption of therapy due to agranulocytosis and the second episode with psychotic symptoms was after a phase of irregular medication. Therefore, the deterioration in this patient cannot be regarded as having become more severe during regular medication. However, in all of the other three patients clinical deterioration occurred in the first months of therapy with D-penicillamine (patients 2 and 4) or with D-penicillamine and zinc sulphate (patient 1) which resolved during futher treatment. In these patients we were able to perform copper measurements during the phases of deterioration. The CSF and the free serum copper concentrations had fallen. Therefore, our findings do not confirm the hypothesis of Brewer et al. [6]. Only in patient 2 was an increase in CSF copper concentration noted 6 months after initial therapy during a phase of continuous clinical improvement. This finding remains unexplained. Possibly copper that is mobilized in the CNS during the first months of treatment gets into the CSF during the further course, leading to an intermittent rise in CSF copper concentration. In all the other patients the CSF copper concentration fell continuously. Further clinical and laboratory examinations might resolve this question.

Definite clinical deterioration during the initial therapy with trien, zinc or thiomolybdate has not been reported so that it might also be a specific problem of therapy with D-penicillamine, However, the number of patients treated initially with zinc, trien or thiomolybdate is small [8, 9, 19] so that it might be possible that such occurrences have not yet been published.

We conclude that further observations over some years confirmed our hypothesis [50] that the CSF copper concentration is a valuable quantitative parameter reflecting the normalization and also the restoring of copper in the CNS in Wilson's disease. Monitoring CSF copper concentration might especially be useful during the initial treatment stages, for an adjustment of therapy to the individual metabolic situation and in the evaluation of new therapeutic strategies.

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