

TETRATHIOMOLYBDATE AS AN ANTIANGIOGENESIS THERAPY FOR SUBFOVEAL CHOROIDAL NEOVASCULARIZATION SECONDARY TO AGE-RELATED MACULAR DEGENERATION

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ABSTRACT

Purpose: Since previous studies have shown that angiogenesis requires copper, this study assessed the efficacy and safety of oral tetrathiomolybdate, an antiangiogenesis drug that binds copper, in subfoveal choroidal neovascularization (CNV) secondary to age-related macular degeneration.

Methods: This phase I trial involved 10 patients with age-related active subfoveal CNV. After patient consent was obtained and initial laboratory tests were performed, patients were given a loading dose of tetrathiomolybdate, followed by a maintenance dose to maintain serum ceruloplasmin (Cp) levels at 5 to 15 mg/dL. Serum Cp levels are a surrogate marker of copper status. Patient follow-up consisted of a detailed protocol that included best corrected visual acuity, measurement of extent of CNV (both classic and occult) on fluorescein angiograms, and laboratory tests to ensure that anemia did not develop. The study was approved by the institutional review board of the University of Michigan Medical Center and by the Food and Drug Administration.

Results: Follow-up of the 10 patients ranged from 4 to 12 months. The targeted serum Cp level was achieved in 8 of the 10 patients. Initially, patients showed stabilization of CNV, but with continued follow-up, all patients showed progression of CNV and loss of visual acuity. Initial mean visual acuity was 20/60; final mean visual acuity was 20/131. At completion of the study, 2 patients showed about a 25% increase in CNV, 1 patient a 60% increase, 1 patient a 100% increase, and 6 patients a 700% to 1,600% increase in CNV.

Conclusion: At the dosages used in this study, tetrathiomolybdate was ineffective in preventing the progression of CNV secondary to age-related macular degeneration.

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INTRODUCTION

Age-related macular degeneration is the leading cause of severe, irreversible visual loss in North America. The Framingham Eye Study¹ showed a 28% prevalence of age-related macular degeneration in participants between the ages of 75 and 85 years. Although the nonexudative form of age-related macular degeneration is more common, the exudative form typically results in severe visual loss. The exudative form is characterized by choroidal neovascularization (CNV), which can be classified as well defined (classic) or poorly defined (occult).²

Current therapies for subfoveal CNV secondary to age-related macular degeneration are very limited. Thermal laser therapy³ can be done, but the therapy destroys the central macula and results in severe visual

loss. Photodynamic therapy⁴ with the drug Visudyne for subfoveal CNV has some efficacy but only for CNV that is predominantly classic.

CNV is a form of angiogenesis that involves a complex series of events, including degradation of extracellular matrix, endothelial cell proliferation, and migration with tube formation necessary for the formation of functional vascular vessels.⁵ Four trials of inhibitors of angiogenesis in CNV secondary to age-related macular degeneration have been completed: isotretinoin,⁶ intravitreal triamcinolone,⁷ interferon alpha-2a,⁸ and matrix metalloprotease inhibitor (Agouron Pharmaceuticals, Inc, San Diego, California, protocol AG3340). None of these trials showed a beneficial effect in controlling CNV secondary to age-related macular degeneration.

Tetrathiomolybdate (TM) is an anticopper drug developed as an orphan therapy for Wilson's disease, an autosomal recessive disorder that leads to abnormal copper accumulation, by Brewer and colleagues.^{9,10} The drug acts by forming a tripartite complex with copper and protein.^{11,12} Given with meals, TM complexes with copper and

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prevents its absorption. Given between meals, TM is absorbed and complexes with serum copper and albumin, rendering the copper unavailable for cellular uptake. This complex is cleared through the kidney and liver. TM is the most potent and most rapidly acting anticopper agent known.

Copper is a major cofactor in angiogenesis.^{13,14} Copper is a required cofactor for the function of many key mediators of angiogenesis, including basic fibroblast growth factor,^{15,16} vascular endothelial growth factor, and angiogenin.¹⁷

Preclinical studies^{18,19} in animal models have demonstrated that TM is an effective antiangiogenic agent in inhibiting the vascularization of mammary carcinoma and squamous cell carcinoma in mice. In a phase I study²⁰ of TM in patients with a variety of solid metastatic tumors, TM-induced copper deficiency achieved stable disease in five of six patients who were copper-deficient at the target range for at least 90 days.

The main toxic effect of TM is a reversible anemia with or without leukopenia, since the bone marrow requires copper for heme synthesis and cell production. This anemia is rapidly reversible by temporarily stopping TM. Extensive experience in treating patients with Wilson's disease and cancer has demonstrated that TM is well tolerated and is an effective anticopper agent in humans. The underlying hypothesis of an anticopper, antiangiogenesis therapy²⁰ is that the level of copper required for angiogenesis is higher than that required for essential copper-dependent cellular functions.

METHODS

A detailed protocol was developed and was approved by the institutional review board of the University of Michigan Medical Center. The study was also approved by the Food and Drug Administration, which limited the study to 10 patients. This study was started prior to the availability of photodynamic therapy with Visudyne.⁴ When photodynamic therapy became available, patients who met the criteria were offered it as an additive therapy.

In this phase I study, serum ceruloplasmin (Cp) was used as a surrogate marker of copper metabolism. Serum copper cannot be used because the complex of TM-copper-albumin is cleared only slowly from the blood and this conjugated copper is measured along with other copper in the serum. Cp is a copper-containing protein, and its synthesis by the liver is regulated by copper metabolism. As copper becomes less available, serum Cp levels begin to decrease, and this is the first sign of copper depletion. Cellular functions dependent on copper are not interfered with as long as there is some Cp in the blood.

The ophthalmic criteria included angiographic evidence of subfoveal CNV secondary to age-related macular degeneration. In seven patients, there had to be some

component of classic CNV but occult CNV could be present. In three patients, the CNV could be entirely occult. A best corrected visual acuity score in the study eye of ≥ 36 using modified EDTRS charts (Snellen equivalent of 20/200) was required. Other criteria included age greater than 50 years, clear media, adequate hematologic test results, natural liver enzyme levels, and willingness to comply with an investigational study. Exclusion criteria included the presence of any concurrent ocular disease that could possibly affect visual acuity, previous photocoagulation involving the center of the macula, foveal scarring, and the presence of any severe or unstable concurrent medical condition or active uncontrolled infections.

Study assessments consisted of best corrected visual acuity with modified ETDRS charts, complete ophthalmic examination, color photographs and fluorescein angiography of the study eye, physical examination, and the following laboratory tests: complete blood cell and platelet counts, serum urea nitrogen, creatinine, electrolytes, liver enzymes, and serum Cp. All visual acuity measurements were performed by a certified visual acuity examiner.

The extent of choroidal neovascularization was measured by a standardized grid placed over the fluorescein angiogram print at a point where the full extent of the neovascularization was evident but before extensive leakage would blur the boundaries of the neovascularization. The standardized grid consists of various circles measuring from 1 through 16 disc areas. Each disc area can be further divided into 0.25 squares and 0.01 squares. All measurements of choroidal neovascularization, both classic and occult, were measured to the nearest 0.01 square.

Induction therapy consisted of 120 mg of TM per day (given as 20 mg three times daily with meals and 60 mg at night), which typically resulted in therapeutic levels in about 4 weeks. Maintenance therapy was adjusted to maintain serum Cp levels at approximately 20% of baseline: 5 to 15 mg/dL. The normal range of serum Cp levels is 16 to 35 mg/dL. Some of our patients began the study with an elevated Cp level, which is usually due to the participation of Cp in the acute phase response to inflammation.

The primary end point was the change from baseline in best corrected visual acuity at 6 and 12 months. A secondary end point was the effect of TM on the extent and morphological changes of choroidal neovascularization as assessed by fluorescein angiography.

RESULTS

The 10 patients consisted of 8 women and 2 men. Their mean age was 72.1 years.

The initial and final best corrected visual acuities, type and extent of CNV, baseline and mean Cp measurements after induction, and duration of follow-up are listed in

Table I. The area of choroidal neovascularization, both classic and occult, is designated in 0.01 squares as outlined in the "Methods" section.

Early in the study, patients showed initial stabilization of CNV with maintenance of good visual acuity, but with longer follow-up, all patients showed progressive loss of visual acuity and continued growth of CNV. At the termination of the study, two patients showed about a 25% increase in CNV, one patient a 60% increase, one patient a 100% increase, and six patients a 700% to 1,600% increase of CNV.

After induction, the mean Cp level was within or very close to the designated therapeutic range of 5 to 15 mg/dL in eight of the patients. In two patients (patients 2 and 8), the mean Cp level was in the mid-20s.

Four patients (1, 3, 6, and 7) who had predominantly classic CNV were also treated with photodynamic therapy with Visudyne in the prescribed manner. In comparing these patients with other nonstudy patients treated only with photodynamic therapy, no additive effect from TM could be discerned. All four patients required additional photodynamic treatments after the study was discontinued.

Complications consisted of a reversible anemia in patient 3, which necessitated that TM be withheld for 4 weeks. The study was stopped about 12 months after the initial patient entered the study. All patients were informed of the study results.

DISCUSSION

Angiogenesis results from a complex interplay of cellular events involving a cascade of factors that are both inhibitory and stimulatory. Surgically excised CNV or post-mortem specimens from patients with age-related macular degeneration have been shown to be immunoreactive to numerous angiogenic factors, including vascular endothe-

lial growth factor,²¹ transforming growth factor-beta,^{1,22} platelet-derived growth factor,²³ and basic fibroblast growth factor.²² Because of the complexity of the angiogenic response, the ideal antiangiogenic therapy¹⁹ would target multiple activators of angiogenesis rather than a single angiogenic factor such as vascular endothelial growth factor. Because copper is a required cofactor for numerous angiogenic mediators,¹⁵⁻¹⁷ copper depletion therapy is an attractive approach to antiangiogenesis therapy. Two animal models^{18,19} have dramatically shown the efficacy of TM therapy as an effective antiangiogenesis therapy in cancerous tumor control. Preliminary trials²⁰ of TM in patients with advanced metastatic disease are encouraging.

Despite this positive background, TM therapy, at the dosages used in this study, was ineffective in preventing the progression of CNV secondary to age-related macular degeneration. The reason for the lack of efficacy is not readily apparent. The majority of patients had Cp levels, reflective of total body copper levels, at the targeted level of 5 to 15 mg/dL. This degree of copper depletion was an effective antiangiogenesis therapy in animal models^{18,19} and in patients with metastatic cancers.²⁰ More profound copper deficiency could possibly have inhibited the progression of the CNV, but greater copper depletion risks more significant potential toxicity.²⁰ Other antiangiogenesis drugs, which were effective in animal models and human tumors, have also been ineffective in age-related CNV. Systemic interferon was effective in an animal model of rubeosis²⁴ and in the treatment of human hemangioma²⁵ but was ineffective in CNV secondary to age-related macular degeneration.⁸

There is evidence that CNV secondary to age-related macular degeneration is particularly resilient and aggressive. Photodynamic therapy⁴ of classic age-related CNV typically results in initial dramatic regression, but the CNV almost universally recurs, requiring multiple treatments to stabilize the disease and typically resulting in severe visual

TABLE I: CLINICAL DATA FOR TEN STUDY PATIENTS TAKING TETRATHIOMOLYBDATE

PATIENT NO.	INITIAL VISUAL ACUITY	FINAL VISUAL ACUITY	TYPE OF CNV	INITIAL EXTENT OF CNV	FINAL EXTENT OF CNV	PDT TREATMENTS	BASELINE CP (mg/dL)	MEAN CP AFTER INDUCTION (mg/dL)	FOLLOW-UP (MO)
1	20/32	20/200	Mainly classic	15	110	1	70.1	15.6	12
2	20/40	20/160	Mainly occult	14	178	0	46.7	27.6	12
3	20/32	20/200	Mainly classic	8	129	2	58.8	14.4	6
4	20/200	20/200	Occult	32	307	0	28.5	13.3	9
5	20/50	20/170	Mainly occult	35	321	0	38.4	11.7	6
6	20/80	20/200	Mainly classic	200	253	1	36.9	6.8	6
7	20/30	20/80	Mainly classic	16	233	1	35.2	18.2	6
8	20/63	20/80	Mainly occult	301	500	0	34.5	24.9	6
9	20/40	20/63	Occult	354	423	0	32.6	11.3	4
10	20/40	20/100	Mainly occult	196	370	0	32.6	11.3	4

CNV, choroidal neovascularization; Cp, ceruloplasmin; PDT, photodynamic therapy.

loss. Four of the patients in this study were treated with photodynamic therapy as an additive treatment while continuing TM therapy. In all four patients, the choroidal neovascularization recurred after initial photodynamic therapy, and multiple photodynamic treatments were required. We could not detect any benefit of TM in preventing the recurrence of the choroidal neovascularization after photodynamic therapy.

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DISCUSSION

DR NORMAN P. BLAIR. Age-related macular degeneration is an increasingly frequent cause of severe, irreversible visual loss. Vine and Brewer are to be congratulated for attempting to improve treatment for choroidal neovascularization, which is usually responsible for this visual loss. None of the current therapeutic options has high efficacy. The authors have taken a rational approach based on the

established involvement of copper in the processes underlying angiogenesis. They have used a potent copper complexing drug, tetrathiomolybdate, which has been shown to be safe by oral administration in human disease. The study was well designed and conducted. They paid attention to admission criteria, visual acuity determination, fluorescein angiogram analysis, and ceruloplasmin measurements.

Why, then, didn't they find evidence of efficacy? First, the power of the study may have been too low with only 10 patients. However, if the efficacy actually had been substantial, one would have expected some suggestion of a benefit. Second, the reduction in copper may have been too small. A reduction in ceruloplasmin to subnormal values was achieved in only 70%, and the average reduction was only to 39%, whereas they had aimed for 20%. Furthermore, it took about four weeks to attain these levels. One wonders whether these goals could be achieved if the drug were administered differently. Third, their hypothesis may not be true. That is, the level of copper required for angiogenesis may not be higher than that required for copper-dependent cellular functions. Fourth, there may have been alternate angiogenic pathways not dependent on copper.

The authors comment that choroidal neovascularization secondary to age-related macular degeneration is particularly resilient and aggressive. While we share in the frustration that leads to this notion, we would take a more sanguine view. As common as it is, choroidal neovascularization never occurs in the majority of people, and it usually occurs only once in those eyes that do develop it. That means that the body has mechanisms to control it. When we obtain a full understanding of choroidal neovascular angiogenesis, it may be possible to devise rather simple and effective therapies. The ultimate way to prevail over this disease will be to understand its stimulus and prevent it. Much work remains before we achieve these objectives, but work such as that of Vine and Brewer represents a significant part of the process.

DR JOHN T. FLYNN. I'd like to suggest that there are a group of diseases at the other end of life, namely in premature infants, where angiogenesis also goes wild and where there are well-developed animal models in the mice, in the rat, and in the kitten. Based on the presentation from the Stem

Cell Symposium today, we should work with the University of Michigan in investigating the use of this drug in those well-defined oxygen-injured animals. We might have a very effective drug for use in premature infants with stage 4 and stage 5 ROP, for which we have no treatment that is effective at the present time.

DR ALFREDO A. SADUN. I have a concern that ethambutol also chelates copper; the mechanism of action is then that it deprives cytochrome oxidase of its proper use and blocks oxidase phosphorylation. This might well be the mechanism by which ethambutol leads to some cases of blindness. It does so by producing a relatively nonabsolute central scotoma and, in the context of this particular disease, the central scotomas, especially without any morphological findings, might well be missed and thus confound the data.

DR ALLAN J. FLACH. This is an uncontrolled study with really no basis for comparison. Looking at the data, which shows a tremendous spread in potential effect, could conceivably be part of a dose response curve of some sort. It's premature to conclude definitively that there is no effect from this therapy.

DR ANDREW K. VINE. I agree with Dr Blair's comment that the hypothesis may well be incorrect for the tissue model we are dealing with. We had looked at the possibility of suppressing copper levels lower but we were unwilling to reduce copper levels any lower because of the significant risk of increased toxicity. The drug is being used in other diseases but we have not considered retinopathy of prematurity, as suppression of angiogenesis in infants would be much more toxic. In terms of the possibility of central scotomas, we have extensive experience with the drug with patients with Wilson's disease. These patients have been documented very thoroughly with visual fields and there is no evidence that these patients developed central scotomas. I agree that our patients would not have been good candidates to pick up on the possibility of an acquired central scotoma. Our results are uncontrolled, but the results do not show any evidence or hint of possible efficacy.

