Copper-Lowering Therapy With Tetrathiomolybdate for Cancer and Diseases of Fibrosis and Inflammation

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Angiogenesis is required for tumor growth and is a likely Achilles heel for cancer. However, antiangiogenic agents have been somewhat disappointing in cancer therapy, perhaps because they target a single angiogenic factor, and there is much redundancy in angiogenic systems. Copper is required for high levels of angiogenesis, and many angiogenic factors have a requirement for copper. Thus, anticopper drugs offer the possibility of more global inhibition. Our group has developed tetrathiomolybdate (TM) for the initial treatment of neurologic Wilson’s disease. Penicillamine makes about 50% of these patients neurologically worse, and many never recover. Only 2 of 55 (3.6%) patients worsened when treated with TM. Because TM exhibited desirable properties of potency, speed, and safety, we studied it as an antiangiogenic agent. We hypothesize that if copper is lowered to midrange, the cellular requirements for copper are met, but angiogenic cytokine signaling is inhibited. TM has shown strong inhibition of cancer growth in five rodent models, encouraging results in a canine study of advanced and metastatic cancer, and encouraging results in a phase 1/2 study of advanced and metastatic cancer in 42 patients. Finally, we have hypothesized that the pathway of fibrosis involving transforming growth factor beta (TGF-β) and connective tissue growth factor is inhibitable by copper-lowering therapy with TM. This pathway is overactive and dysregulated in many diseases of fibrosis. In animal studies, TM has completely inhibited the pulmonary fibrosis induced by bleomycin, the hepatitis induced by concanavalin A, and the cirrhosis induced by carbon tetrachloride. We find that TM inhibits transforming growth factor beta and inflammatory cytokines tumor necrosis factor alpha and interleukin-1-beta. J. Trace Elem. Exp. Med. 16:191–199, 2003. © 2003 Wiley-Liss, Inc.

Key words: antiangiogenesis; anticopper drugs; Wilson's disease; cytokines; transforming growth factor beta; tumor necrosis factor alpha

Contract grant sponsor: US Food and Drug Administration’s Orphan Products Office; Contract grant numbers: FD-R-000179 and FD-U-000505; Contract grant sponsor: National Institutes of Health; Contract grant number: MO1-RR00042; Contract grant sponsor: Attenuon LLC, San Diego, CA.

The University of Michigan has recently licensed the antiangiogenic uses of TM to Attenuon LLC, and Dr. Brewer has equity in Attenuon LLC.

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Received 4 February 2003; Accepted 16 July 2003

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INTRODUCTION

This work stems from a presentation at the sixth meeting of the International Society for Trace Element Research in Humans held in Quebec City, Canada, September 7–12, 2002. It was the leadoff presentation of a symposium on copper and angiogenesis and in it I gave a brief overview of the topics of angiogenesis and cancer and copper and angiogenesis at the beginning of the session before getting into the research topics of the presentation.

ANGIOGENESIS AND CANCER

Folkman [1–3] pioneered the concept that tumor growth requires angiogenesis and that this requirement might be an Achilles heel for cancer because in adults there is little requirement for angiogenesis. The concept of antiangiogenic therapy for cancer has taken off in the last decade [4,5], although it is fair to say that results so far have not met previous high expectations, particularly when used as monotherapy [6–8]. A major reason is that angiogenesis has turned out to be a very complex area, with dozens of proangiogenic factors and angiogenic inhibitors potentially interacting [4,9]. It seems likely that therapeutic inhibition of one angiogenic factor or its receptor is simply overcome by the tumor recruiting other factors to overcome the block in angiogenesis produced by the therapy. Thus, current concepts are directed towards finding a global inhibition of angiogenesis produced by the therapy, combining multiple antiangiogenic agents, or combining antiangiogenic therapy with other modalities, such as chemotherapy. This last is based on the logical concept that if tumors are reduced to the smallest possible size by treatment with a modality such as chemotherapy, there is an increased probability that antiangiogenic therapy will prevent regrowth. That is, the fewer the cancer cells remaining, the less likely the cancer will find mutational or other mechanisms for recruiting angiogenic promoters insensitive to the agent being used.

COPPER AND ANGIOGENESIS

The involvement of copper in angiogenesis has been known for a couple of decades. Copper, or copper-containing molecules, such as ceruloplasmin, when placed in the cornea of the rabbit eye, stimulated angiogenesis [10,11]. If rabbits were made copper deficient with a combination of penicillamine and a low-copper diet, angiogenic substances placed in the cornea, such as prostaglandin E₂, were much less angiogenic [12]. Brem et al. transplanted brain tumors into brains of rats and rabbits and saw much less tumor growth and tumor invasion of normal tissue in copper-deficient animals than in controls [13,14].

ANTICOPPER DRUGS

The three anticopper drugs that are commercially available are penicillamine, trientine, and zinc. They were all developed as therapies for Wilson’s disease, an
inherited disease of copper accumulation and copper toxicity. Patients with this
disease must be on some type of anticopper drug for the rest of their lives to get
rid of excess copper and prevent its reaccumulation [15,16].

Penicillamine is the oldest, introduced by Walshe in 1956 [17]. It is a reductive
chelator that is quite effective in mobilizing copper and causing large amounts of
copper to be excreted in the urine. It is an effective treatment for Wilson’s disease
but unfortunately has a long list of side effects [15,16]. It also has a major
disadvantage in that if used in Wilson’s disease patients presenting with neuro-
logical symptoms, it makes 50% of them worse, probably by increasing brain
copper in the process of mobilizing the large hepatic stores of copper [18]. Half of
the patients who worsen, or 25% of the original sample, never recover to their
prepenicillamine baseline; in other words, 1 in 4 of these patients end up with
additional, penicillamine-induced, permanent disability.

Trientine was developed by Walshe [19] as a replacement for penicillamine in
Wilson’s disease patients who were intolerant of penicillamine. It has a similar
mechanism of action as penicillamine, acting as a chelator to enhance urinary
excretion of copper, but is a much less aggressive drug. It has not been studied
very thoroughly, but seems to be generally effective in Wilson’s disease. It shares
the same toxicities as penicillamine but at a reduced frequency [15,16]. I will
discuss its use in Wilson’s disease below.

Zinc has been developed by my group [15,16,20] and was approved for
maintenance use in Wilson’s disease by the Food and Drug Administration in
1997. Historically, it was first used by Schouwink in two patients in the Neth-
erlands, but the findings were never published in the general literature [21]. Our
use in Wilson’s disease was stimulated by our observation that zinc therapy in
sickle cell anemia produced copper deficiency as a side effect [22–24]. Zinc acts
by inducing intestinal cell metallothionein, which has a high affinity for copper,
and blocks copper transfer from the intestine into the blood [25]. The intestinal
cells slough with a 6-day turnover time and take the metallothionein copper
complex into the stool.

**DEVELOPMENT OF TETRATHIOMOLYBDATE FOR WILSON’S DISEASE**

As we worked with zinc to develop it as a maintenance therapy, we became
aware, from the histories of our patients, of the problem with penicillamine in
making neurologically presenting patients neurologically worse [18]. Thus,
penicillamine was contraindicated in these patients. We believe zinc is two slow
acting for acutely ill, copper toxic, patients. Trientine had not been tried in these
patients, but because it shared penicillamine’s mechanism of action, we were
concerned that it would precipitate initial worsening.

The lack of a drug for initial therapy led us to develop tetrathiomolybdate
(TM) for this purpose [26–29]. TM had, based on animal studies, the appro-
priate qualities of fast action and lack of toxicity. It had been tried, briefly, as
maintenance therapy in Wilson’s disease but had been given up because the
patients developed anemia [30]. The mechanism of action in TM involves a
tripartite complex with protein and copper [31–35]. Given with food, TM forms
a complex with food protein and copper and prevents copper absorption. Given
without food, TM is absorbed into the blood and complexes available copper
(potentially toxic copper) with albumin. This copper is unavailable for cellular
uptake and is thus nontoxic.

Our first trial was an open study that accrued 55 patients [29]. The design
included an 8-week admission to the General Clinical Research Center of the
University of Michigan Hospital, during which the patient received 20 mg of TM
three times daily with meals and 60–350 mg of TM away from food. The pa-
tients were followed with a semiquantitative neurological score (0–38, with 0
normal) and a semiquantitative speech score (0–7, with 0 normal). A consistent
deterioration of 5 on the neurology score or a deterioration of 2 on the speech
score was considered significant deterioration.

Only 2 of the 55 patients reached our criteria, or 3.6%, compared with the
roughly 50% that worsened on penicillamine therapy [29]. Over the next 2 years,
during which the patients were on maintenance zinc therapy, they showed sub-
stantial neurologic recovery.

We observed two side effects in this study [29]. One was anemia, sometimes
accompanied by leukopenia, in six patients. The other was mild further elevation
of transaminase enzymes, which occurred in three patients. Both side effects
tended to occur more frequently with higher doses or more rapid dose escalation.
The anemia/leukopenia is attributed to overtreatment and regional depletion of
copper in the bone marrow. The reason for the transaminase elevations is un-
known. However, we have not observed it in TM use in cancer patients, so we
suspect it is related to an interaction with copper in the copper-loaded livers of
these patients. Both side effects respond quickly to a dose reduction or drug
holiday.

Because trientine had never been studied for use as initial therapy in neuro-
logically presenting patients and because it is a less aggressive drug than peni-
cillamine, we have conducted a double-blind study comparing the two drugs in
this type of patient. This study is almost finished. The design involved 8 weeks of
therapy with either 500 mg of trientine twice daily or 20 mg of TM 6 times daily,
three of the doses with meals. Both drugs were combined with zinc 50 mg twice
daily. The primary measures were the neurologic and speech scores described
above, evaluating the frequency of neurologic deterioration. This study is just
being finished, but a preliminary examination of the data indicates a deteriora-
tion rate with trientine of between 20 and 25%, and confirmation of a low
deterioration rate with TM.

In this last study, the frequency of side effects with TM was about 13% thus
far for the anemia/leukopenia and 17% for the elevated transaminase enzymes.
Both side effects responded quickly to halving the dose. We are now initiating a
double-blind comparison of 120 mg of TM daily for 8 weeks, the regimen we
have been using, to 60 mg of TM daily for 16 weeks, hoping to retain the
excellent efficacy and reduce or eliminate the side effects.

Finally, we are evaluating TM for the initial treatment of Wilson’s disease
patients presenting with hepatic failure, in a double blind, three-arm, comparison
with penicillamine and trientine.
CANCER THERAPY STUDIES WITH TETRATHIOMOLYBDATE

As we developed TM for Wilson’s disease and discovered its excellent properties of anticopper potency, rapid action, and low toxicity, we became interested in evaluating TM as an antiangiogenic anticancer agent based on the copper lowering rationale inhibiting angiogenesis discussed earlier. We first used a mouse model of MCA 205 sarcoma cells, injected subcutaneously into the flank, and demonstrated a significant effect of TM on slowing growth rate and on decreasing the size of the tumor at sacrifice (Brewer, unpublished data). This led us to team up with an oncologist, Dr. Sofia Merajver, who designed an elegant study using the HER2/neu transgenic mammary cancer genetic model. These mice all develop mammary cancer over the first year of life. Half of the mice were treated with TM, half with vehicle. Over 218 days, 11 of 22 control mice developed obvious tumors, while none of 15 TM mice developed detectable tumors [36]. Release from TM treatment of a few mice resulted in tumor development in all cases. Histologic examination of the breasts of TM treated mice revealed small, avascular, tumor masses.

Our team has gone on to develop positive therapeutic results with TM in four other mouse tumor models [36–39], in a phase 1/2 study of spontaneous advanced and metastatic cancer in pet dogs (Kent, Madewell, and Brewer, unpublished data), and in a phase 1/2 study of advanced and metastatic cancer in human patients [40]. Further details of these studies will be provided in other papers published from this symposium.

ANTIFIBROTIC AND ANTIINFLAMMATORY STUDIES WITH TETRATHIOMOLYBDATE

The antiangiogenic mechanism of action of TM in cancer appears to involve inhibition of several proangiogenic cytokines and inhibition of nuclear factor-kappa B (NF-κB), a master switch for many cytokines [36]. The pathway of fibrosis, physiologically important in wound repair and growth and development, consists of a series of cytokines in which transforming growth factor beta (TGF-β) and connective tissue growth factor play key roles. This pathway becomes dysregulated and overactive in diseases of fibrosis involving many organs, such as the lung, liver (cirrhosis), kidney, and skin (scleroderma) [41–47]. In examining the cytokines of this pathway, it seemed likely that the pathway might also be copper dependent. For example, connective tissue growth factor is rich in cyssteine, often a tip off of copper binding [45]. One of the activators of TGF-β is SPARC (secreted protein acidic and rich in cysteine), already known to be copper dependent [48–50].

To test these ideas, we worked with Dr. Sem Phan, a pathologist at the University of Michigan. We used the bleomycin mouse model. In this model, bleomycin instilled into the trachea produces an inflammatory reaction peaking in 7 days, with tumor necrosis factor alpha (TNF-α) playing a major role, and leads to extensive pulmonary fibrosis by 21 days, which is when the mice were sacrificed. Fibrosis can be measured by the level of hydroxyproline, a major
constituent of collagen, in the lung, as well as visualized histologically. The increasing pulmonary fibrosis produces an illness in the mice, easily followed by weight loss.

TM therapy initiated before bleomycin injury completely prevented the fibrosis as measured by hydroxyproline and as seen in histological sections [51,52]. It also completely prevented the illness as evaluated by completely preventing the weight loss from bleomycin injury. At day 7, marked inhibition of TNF-α and TGF-β messenger-RNA production by TM was found in mouse lung, as well as a reduction in TGF-β protein (Brewer and Phan, unpublished data). Furthermore, TM treatment could be withheld until several days after bleomycin injury, such that copper depletion wouldn’t occur until after the 7 days when the inflammatory reactions peaks, and still produce a significant reduction in hydroxyproline levels [52]. The 7-day experiment shows that we are inhibiting inflammation, possibly through inhibition of TNF-α. This last experiment shows that we are directly inhibiting fibrosis, not just inhibiting inflammation, which then leads to less fibrosis. We have gone on and in collaboration with Dr. Fred Askari, a hepatologist at the University of Michigan, and shown that we can be similarly successful in inhibiting hepatic injury with TM from either concanavalin A or carbon tetrachloride [53].

We interpret these results in animal model studies to mean that TM may be effective against a large number of fibrotic and inflammatory conditions. A partial list of such therapeutic targets, to exemplify the wide range of possibilities is given in Table I. To begin testing whether the positive animal results carry over to human patients, we are initiating a clinical trial of TM in idiopathic pulmonary fibrosis, with Dr. Kevin Flaherty, a pulmonologist at the University of Michigan, as the principal investigator.

**SUMMARY**

Angiogenesis is critical to cancer growth, but antiangiogenic agents as monotherapy targeting specific angiogenic agents or their receptors have not been table I. Partial List of Possible Therapeutic Targets of Tetrathiomolybdate Based on Antifibrotic and Antiinflammatory Effects

<table>
<thead>
<tr>
<th>Acute Respiratory Distress Syndrome</th>
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<tr>
<td>Idiopathic Pulmonary Fibrosis</td>
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<tr>
<td>Various Types of Liver Injury</td>
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<tr>
<td>Various Types of Cirrhosis</td>
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<td>Various Types of Renal Injury</td>
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<tr>
<td>Various Types of Renal Fibrosis</td>
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<tr>
<td>Scleroderma</td>
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<td>Disabling Keloid</td>
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<td>Inflammatory and Fibrotic Diseases of the Gastrointestinal Track</td>
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<tr>
<td>Rheumatoid Arthritis</td>
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<td>Autoimmune (Connective Tissue) Disorders</td>
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<tr>
<td>Various Other Diseases of Fibrosis and/or Inflammation</td>
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extremely effective as anticancer agents, probably because of the large number of angiogenic promoters, allowing the tumor to take advantage of this redundancy. Anticopper agents have some promise as more global inhibitors of angiogenesis because of the copper dependence of many angiogenic factors. TM as an anticopper agent in animal tumor models looks very effective. In limited patient trials of advanced and metastatic cancer, it has shown encouraging results as sole therapy, but will probably be much more effective in combination with agents, such as chemotherapy, which greatly reduce the tumor burden at the beginning.

TM has shown surprising and dramatic efficacy in lung and liver models of injury which normally produce inflammation and fibrosis. Copper-lowering therapy with TM appears to inhibit inflammatory and fibrotic cytokines which become dysregulated and overactive in a large number of diseases of inflammation and fibrosis. Clinical trials are beginning to see if this aspect of TM efficacy carries over from animals to humans.

ACKNOWLEDGMENTS

In all of the work described in this article, where data were derived from human patients, the studies were approved by the University of Michigan Institutional Review Board, and informed, written consent was obtained from each patient.

REFERENCES

49. Strandjord TP, Madtes DK, Weiss DJ, Sage EH. Collagen accumulation is decreased in SPARC-null mice with bleomycin-induced pulmonary fibrosis. Am Physiol Soc 1999;L628–L635.