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A copper-lowering strategy attenuates amyloid pathology in a transgenic mouse model of Alzheimer's disease

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Abstract

There is increasing evidence for the crucial role of metals in the pathology of Alzheimer's disease. Both the aggregation and neurotoxicity of beta amyloid are dependent on the presence of copper. This study investigated the ability of the copper-complexing drug tetrathiomolybdate to reduce beta amyloid pathology and spatial memory impairment in both a prevention and a treatment paradigm in the Tg2576 mouse model of Alzheimer's disease. Tetrathiomolybdate treatment lowered brain copper and reduced beta amyloid levels in the prevention paradigm, but not in the treatment paradigm. Our data suggests that controlled lowering of systemic copper may achieve anti-amyloid effects if initiated early in the disease process.

Keywords

Alzheimer's disease; tetrathiomolybdate; Tg2576; amyloid; copper

INTRODUCTION

The pathological features of Alzheimer's disease (AD) include deposition of beta amyloid (A β) plaques and neurofibrillary tangles in the cortex and hippocampus. A large body of evidence suggests that copper plays a multifaceted role in AD pathology. The complex role of copper is implicated by the findings that brain levels of copper increase with aging [1], amyloid precursor protein (A β PP) expression is up-regulated by copper [2], and the aggregation and neurotoxicity of beta amyloid (A β) are dependent on the presence of copper in vitro [3–6] and in vivo [3]. These findings suggest that interventions that lower brain copper levels may also reduce A β pathology and its consequences.

The copper binding drug, clioquinol, which crosses the blood brain barrier (BBB), has been shown to lower brain A β pathology in a transgenic mouse model of AD, but this occurred without lowering brain copper levels [4]. A small pilot clinical trial of clioquinol was

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encouraging [5], but clioquinol has not advanced to large-scale clinical trials because of technical issues surrounding GMP manufacture of the drug. Concerns about neurotoxicity in human subjects have also been raised [6]. Consequently, alternative copper-complexing agents suitable for use in human subjects are consequently of interest, and clioquinol analogs are in development [7,8].

In AD, copper derangement is characterized by extracellular excess and intracellular deficiency [9]. For this reason, copper-modifying strategies are currently focused on drugs that cross the BBB and “correct” this copper redistribution. There is modest evidence supporting the contention that a copper-complexing agent must cross the BBB to be effective in modulating A β pathology. For example, the copper chelator trientine (which does not cross the BBB) did not lower brain A β in Tg2576 mice in the same experimental paradigm in which clioquinol was effective [4]. Since neither clioquinol nor its analog PBT2 [7,8] affect plasma and brain copper levels, these agents may act by crossing the BBB and directly interfering with copper binding to A β rather than by lowering systemic copper levels. By promoting the uptake of zinc and copper, Clioquinol and PBT2 have also been shown to have positive effects on cognition and A β clearance by virtue of their ability to redistribute and correct unbalanced metal homeostasis [7,10–15]. Nevertheless, several studies suggest that excess systemic copper is associated with AD [16]. We hypothesized that controlled-lowering of systemic copper levels would reduce brain copper, lower brain A β , and lower A β neurotoxicity. We tested this hypothesis by chronically treating the Tg2576 mouse model of AD with the copper-complexing agent tetrathiomolybdate (TM). TM is a copper-binding agent that has been used as an investigational agent in human subjects with Wilson’s disease as well as other copper overload conditions. Although the BBB penetration of TM is not established, the ability of TM to ameliorate neurologic impairment has been demonstrated in Wilson’s disease [17,18]. We tested the ability of TM to attenuate A β pathology and spatial memory impairment in both a prevention and a treatment paradigm in Tg2576 mice. Plasma ceruloplasmin (Cp) levels were used as a surrogate marker of circulating free copper to titrate the dose of TM and ensure systemic copper-lowering without inducing overt copper deficiency. The deliberate copper-lowering aspect of this strategy is distinct from the approach with clioquinol and its derivatives, and consequently may point to alternative copper-modulating strategies for clinical trials in AD.

MATERIALS AND METHODS

Animals and diet

Tg2576 mice were generated from a breeding pair generously provided by Dr. Karen Hsiao-Ashe (Mayo Clinic, MN). The transgene was carried on a genetic background of C57BL/6J X SJL. Following weaning, litters were genotyped and group-housed (4–5 per cage) until the commencement of experiments. All of the mice in this study were female because male Tg2576 mice are prone to marked aggression towards cage mates. Mice were maintained in a climate-controlled environment with a 12-hr light/12-hr dark cycle, and fed AIN-93M Purified Rodent Diet (Dyets Inc, Bethlehem, PA). Diet and water were supplied ad libitum. All procedures were conducted in accordance with the NIH Guidelines for the Care and Use of Laboratory Animals and were approved by the institutional Animal Care and Use Committee of the Portland VA Medical Center.

Treatments

Tg2576 and wild type mice were randomized to receive either TM or vehicle in their drinking water. TM doses were adjusted on a weekly basis in order to assure that body weight did not drop more than 10%. In the prevention paradigm, TM was administered at an average dose of 9.2 mg TM/L vehicle in their drinking water (vehicle = 9.2 mg sodium bicarbonate/L). In the

treatment paradigm, TM was administered at an average dose of 12.5 mg TM/L vehicle (vehicle = 12.5 mg sodium bicarbonate/L drinking water). In both paradigms, control mice received vehicle only. In the prevention paradigm, 7–10 month-old mice were treated for six months as follows: 30 wild-type littermate controls (vehicle n=16, TM n=14) and 28 Tg2576 mice (vehicle n=14, TM n=14). In the treatment paradigm, 12 month-old mice were treated for six months as follows: 24 wild type mice (vehicle n=11, TM n=13) and 14 Tg2576 mice (vehicle n=7, TM n=7).

Ceruloplasmin assay

Blood was collected from the saphenous vein every 2–3 weeks from 4–6 animals per treatment group and plasma Cp activity measured using a modified version of the oxidase method [19]. Briefly, for each mouse, 7.5 μ l of plasma and 75 μ l of 100 mM sodium acetate (pH 6.0) were added in duplicate to a 96-well plate and incubated 5 min at 37°C. Aqueous o-dianisidine dihydrochloride solution (30 μ l, 2.5 mg/ml, pre-incubated to 37°C) were added to each well and the plate incubated at 37°C for 5 min. An aliquot (56.3 μ l) of the mixture was immediately transferred to empty wells. The solution remaining in the original wells (t=5 min) was quenched with 150 μ l 9M sulfuric acid. The plate was incubated for an additional 15 min at 37°C, then the secondary wells (t=20 min) were quenched. Absorbance was read at 540 nm and the enzymatic activity of ceruloplasmin determined from the following equation: Activity (U/ml) = (Abs_{t=20} - Abs_{t=5})(416)

Behavioral analysis

In order to monitor for potential neurologic toxicity, locomotor performance was assessed using rotarod. Testing was performed using a customized rotarod (Med Associates, Georgia, VT) with a 2 cm dowel and four lanes for mice. Mice were placed on the dowel in their respective lanes and the dowel started at 4 RPM and gradually and uniformly accelerated to 40 RPM over 300. Falls were captured by an electric beam. Each mouse received ten trials per day for two consecutive days and performance was expressed as duration on the rod.

Because increased open field activity has been reported in the Tg2576 model [20], we evaluated the effects of TM on this behavior. Each mouse was placed in a square arena (38 × 38 × 64 cm high, constructed of white acrylonitrile butadiene styrene) for two 5 min open field sessions on each of three consecutive days. A camera mounted above the arena, interfaced with ANY-maze video tracking system (Stoelting Co, Wood Dale, IL) captured the total distance moved (cm), velocity (cm/sec) and thigmotaxis.

To assess hippocampal-dependent spatial learning and memory, mice were trained in a standard Morris water maze task using the same apparatus and protocol used in our previous studies [18,19]. Briefly, a cylindrical tank (109 cm in diameter) constructed of seamless white high-density polyethylene was positioned in a testing room surrounded by various distinct extra maze cues. The tank was filled to a depth of 33 cm with water rendered opaque by the addition of non-toxic Tempa paint. A 13 cm diameter escape platform was constructed of clear Plexiglass. All behavior was acquired by ANYmaze videotracking software. To verify that poor performance on the hidden platform task results from cognitive impairments rather than a deficit of vision, motivation or locomotor function, mice first underwent visible platform training. Mice were trained to swim to a plexiglass platform positioned 1 cm above the water surface. Mice were trained on the hidden platform for 16 trials (8/day). 24 h after hidden platform training, mice were spatially trained on the hidden platform water maze task. Extra-maze cues were positioned on floor to ceiling curtains. Mice were trained for 40 trials (8/day) to learn the location of a escape platform submerged 1 cm below the water surface in the center of the NE quadrant of the pool. The platform position remained fixed throughout training. During a given trial, the mouse was introduced into the pool, at one of four pseudorandomly-

chosen start points (N,S,W,E) and allowed 60s to find the platform. If the mouse did not find the platform after 60s, it was placed on the platform. The mouse remained on the platform for 30s before being returned to a holding cage for an inter-trial interval minimum of 7 min. Escape latency (in s) was used as a measure of spatial learning. For spatial memory testing, a 60 s free swim probe test, in which the escape platform was not present in the pool, was conducted 24 h after the final spatial training trial. Search behavior during the probe test was analyzed to determine (i) the percent dwell in each quadrant (ii) the number of crossings into a circular zone in the quadrant encompassing the previous location of the platform and (iii) the average distance of the mouse to the platform (in cm) during the probe test. A ratio of the number of crossings into the training zone quadrant to the total number of crossings into all four quadrants was generated. Search ratios greater than 25% indicate a bias towards the training quadrant, while a ratio less than 25% reflects a lack of preferential searching.

Tissue homogenates and ELISA

Following behavioral analysis, animals were euthanized by CO₂ inhalation and cervical dislocation. Blood and liver samples were collected and frozen. Brains were quickly removed and divided. The anterior 3 mm of bilateral frontal cortex and a 1 mm section behind the frontal cortex (“frontal slice”) were dissected and frozen for A β ELISA and brain metal analysis respectively. The right hemisphere was immersion fixed in 4% formaldehyde in phosphate buffered saline for histochemical analysis. The contralateral hemisphere was frozen for subsequent isolation of RNA and determination of copper-dependent enzyme activity.

Frontal cortex tissue was homogenized in tris buffered saline (TBS) containing protease inhibitor cocktail (EMD Chemicals, Gibbstown, NJ), then centrifuged at 100,000 $\times$ g for 30 min at 4C. The supernatant from this step was labeled “fraction A, cytoplasm”. The pellet generated in this step was homogenized in TBS, protease inhibitors and 1% Triton X-100 (Roche, Indianapolis, IL), centrifuged at 100,000 $\times$ g 30 min, and the supernatant collected and labeled “B, soluble membrane fraction”. The pellet generated following the second centrifugation was homogenized in buffer containing TBS, 1% N-lauroylsarcosine sodium salt (Sigma, St. Louis, Mo) and protease inhibitors, then centrifuged at 100,000 $\times$ g for 30 min. The resulting pellet was collected, washed in TBS containing protease inhibitors and re-centrifuged. The final pellet was homogenized in 70% formic acid (Sigma), incubated for 1 h at room temperature, and centrifuged at 100,000 $\times$ g 30 min. The resulting supernatant was collected and labeled “insoluble fraction”. A β 1–40 and A β 1–42 were measured in the soluble membrane and insoluble fractions using commercial ELISA (Invitrogen, Camarillo, CA) according to the manufacturer’s instructions.

Immunohistochemistry

Forty micron frozen coronal sections were cut on a freezing microtome. Sections were incubated with agitation in blocking buffer (100 mM TBS, pH 8.0, 2 mg/ml bovine serum albumin, 2% horse serum, 0.5% Triton X-100) for 2h, then incubated overnight with primary antibody diluted 1:1000 in blocking buffer (rabbit polyclonal antibodies directed against either A β or human A β PP (Invitrogen). Sections were then incubated for 2 h with biotinylated secondary antibody (1:200, Vector Labs, Burlingame, CA), for 2 h with an avidin-linked peroxidase complex (ABC, Vector Labs), then developed with diaminobenzidine (DAB, Sigma) in PBS. Sections were washed, mounted in Permount (Fisher Scientific, Pittsburg, PA) and cover slipped. Protein expression was quantified in at least three coronal sections from each mouse, representing anterior, middle and posterior hippocampus and cortex. Hippocampal and cortical areas were traced using a computerized stage and stereo investigator software (Image J, Wayne Rasband, NIH, USA). A β and A β PP levels were expressed as percentage of hippocampus or cortex occupied by these proteins. Mean values for each parameter were calculated from at least three sections per animal.

Copper-dependent proteins

Copper-dependent enzyme activity was measured in left hemisphere homogenates (cytoplasmic fraction A) from the prevention cohort. Cytochrome c oxidase activity was determined by measuring the oxidation of ferricytochrome c using the Cytochrome c Oxidase Assay Kit (Sigma, St. Louis, Mo) following manufacturer's instructions. Cytochrome c oxidase and its inhibitor sodium azide were respectively used as positive and negative controls. Superoxide dismutase (SOD) activity was determined using the NWLSS Superoxide Dismutase Activity Assay (Northwest Life Science Specialties, LLC, Vancouver, WA) according to manufacturer's instructions. This assay is specific for Cu/Zn, Mn and Fe isoforms of SOD, however Cu/Zn SOD is the most common cytoplasmic form of this enzyme.

Gene expression

RNA was isolated from left hemisphere sections using Ambion RNAqueous. Relative mRNA expression was determined using quantitative real time PCR using the Taqman Gene Expression Assay with commercially-available primer/probe sets for murine amyloid precursor protein, beta secretase, presenilin, and glyceraldehyde-3-phosphate dehydrogenase (GAP). Plates were analyzed on a StepOnePlus Real Time PCR system using the ddCt method. All reagents were from ABI (Applied Biosystems, Foster City, CA). Because expression of human A β PP is driven by a non-physiological promoter and is over-expressed in the Tg2576 model, we did not measure the effects of TM treatment on human A β PP.

Tissue metal assays

Liver and brain zinc, iron, copper and molybdenum levels were measured using atomic absorption spectroscopy (AAS). Tissues (approximately 20 - 100 mg wet weight) were digested using a modified version of Parker et al. [21]. Briefly, samples were incubated overnight in 0.5 - 2 ml of concentrated nitric acid (~69.5%), centrifuged at 12,000 $\times$ g for 5 min and the supernatants sequentially diluted to yield 2.5–8 ml of solutions containing 35% nitric acid. AAS measurements were carried out using an AA6650 spectrometer (Shimadzu, Columbia, MD) equipped with a graphite furnace and ASC6100 autosampler. Samples were diluted with 2% nitric acid to be in the linear absorption range of the calibration curve (1–10 ppb (ng/L)). Twenty μ l of each sample was injected up to 3 times depending on the standard deviation of measurements. Copper and molybdenum concentrations were derived by comparing the absorption of the samples with a standard curve.

Western blot Analysis

A β PP Protein expression in the soluble membrane fraction from the prevention cohort was determined by western blot analysis. Briefly, protein quantity in each sample was determined using the BCA assay (Pierce). Protein (20 μ g) was loaded onto 4–12% Bis-Tris gels, then separated and transferred onto nitrocellulose using the NuPage® electrophoresis system (Invitrogen). The membrane was probed with antibodies to A β PP (Biosource) and β -tubulin (Sigma), treated with appropriate secondary antibody, exposed to enhanced chemiluminescence solutions (Invitrogen), visualized using the Molecular Imager Gel Documentation System and quantified using Quantity One Software (Bio-Rad).

Statistics

Data were analyzed using GraphPad Prism 5 (GraphPAD Software Inc) using one-way analysis of variance followed by the Bonferroni post-hoc test (for comparisons between two samples at a time).

RESULTS

TM was titrated to target plasma ceruloplasmin levels

Based on animal studies of TM for conditions such as Wilson's disease and advanced cancers [22], a target plasma Cp of 30–60% of vehicle-treated animals was set. After initial titration, the target Cp level was achieved and maintained in both the prevention and the treatment experiments. Doses of TM required to achieve target Cp levels (9–12.5 mg TM/L) were slightly lower than those used in other mouse models of diseases such as multiple sclerosis and cancer (15–20 mg TM/L) [23,24]. Over the course of treatment, average Cp for TM-treated animals was $35 \pm 8\%$ of vehicle for the prevention trial (Fig 1a) and $58 \pm 4\%$ for the treatment cohort (Fig 1b). Genotype had no effect on Cp values (not shown) therefore the data in Fig 1 reflects Cp values for both wild type and transgenic animals.

Brain copper levels were significantly lowered by chronic TM treatment

In both the prevention (Fig 2a) and treatment experiments (Fig 2b), cortical copper levels were significantly reduced in mice treated with TM compared to vehicle-treated mice. Additional studies measuring zinc, and iron in TM-treated and control brain tissue confirmed that the metal lowering effect was specific to copper (data not shown).

TM levels were increased in the livers of the treated animals, but not in the brain

Both copper (Fig 3a) and molybdenum (Fig 3b) levels increased in the livers of TM-treated animals. Molybdenum was measured as a marker of TM. Molybdenum levels in the brains of vehicle and TM-treated animals and livers of vehicle-treated animals (Fig 3b) were below the level of detection. Taken together, this suggests that TM does not accumulate in the brain.

TM treatment was well tolerated

In addition to Cp, changes in body weight were used as criteria to evaluate potential toxicity during the course of treatment. TM doses were adjusted on a weekly basis to assure that body weights did not drop more than 10%. Over the course of treatment, the average body weights were 94% and 98% of vehicle-treated animals for the prevention and treatment cohorts, respectively. Using values for water consumption, animal weights and TM concentrations, we calculated that the TM prevention cohort received an average daily dose of 1.1 mg TM/kg body weight and the treatment cohort received an average daily dose of 1.3 mg TM/kg body weight. There were no significant effects of TM treatment upon survival or brain activity of the copper-dependent enzymes cytochrome c oxidase (Fig 4a) or superoxide dismutase (Fig 4b). TM did not exhibit neurologic toxicity (evaluated by rotorod performance) or affect general locomotor activity (measure by open field activity) in either the prevention or treatment cohorts. Results for rotorod (5a) and open field (5b) performance are shown for the prevention cohort. As previously reported [17], increased open field activity was observed in Tg2576 mice.

TM significantly reduced Insoluble brain A β in the “prevention” paradigm

In the prevention experiment, TM had no effect on soluble levels of A β 1–40 as shown by ELISA (Fig 6a). Soluble A β 1–42 was undetectable. Insoluble cortical A β 1–40 and A β 1–42 were significantly reduced (Figs 6b&c). Histological A β burden was also significantly reduced in the cortex, with a favorable trend seen in the hippocampus (Figs 6d&e).

In the treatment experiment, TM treatment significantly reduced soluble A β 1–40 (Fig 7a), but the levels of insoluble A β 1–40 and A β 1–42 were not significantly changed (Figs 7c&d). There was also no effect on histological A β burden (Fig 7e).

TM did not affect A β PP processing

In order to determine whether TM lowered A β in the prevention cohort by affecting the processing of A β PP, the expression of β -secretase (BACE), presenilin (PSEN), murine and human A β PP were measured by quantitative real time PCR. TM had no effect on the levels of these proteins (Fig 8a). TM also had no effect on A β PP expression, as illustrated by western blot analysis (Figs 8b & c).

TM treatment was not associated with an improvement in spatial memory

At the end of treatment, the Morris water maze was used to assess the effect of treatment on spatial memory. TM treatment had no effect on spatial memory in either cohort. Representative results are shown for the prevention trial. There were no significant genotype or treatment differences in performance on the hippocampal-independent visible platform water maze task (Fig 9a). Mean escape latencies per each four-trial block of hidden platform is presented in Fig 9b. Tg2576 mice exhibited impaired spatial memory compared to wild type mice ($p < 0.05$ after trial 6), however TM treatment had no effect on this task. In the probe test measurement of spatial memory retention, all mice showed a statistically significant preference for the target quadrant ($p=.001$, Fig 9c). Neither genotype nor treatment affected this task.

DISCUSSION

These data show that controlled lowering of systemic copper, monitored in a manner that may be readily translated to human subjects, results in attenuation of AD-type brain pathology when treatment is initiated prior to the appearance of pathology. The absence of measurable molybdenum in the brains of TM-treated animals suggests that the copper lowering and anti-amyloid effects in the brain were achieved in the prevention paradigm without delivering drug across the BBB. In contrast, the amyloid-lowering effect was not present in the treatment paradigm despite TM lowering plasma Cp levels and reducing cortical copper levels in both experiments. Both groups were treated for six months with TM and it is possible that this discrepancy is due to the older age of the treatment cohort. Given that amyloid burden is age-dependent, the inability of TM to lower amyloid levels in the older treatment cohort may be attributed to a saturation of endogenous A β clearing systems. Alternatively, TM may be able to prevent but not reverse copper-dependent amyloid aggregation because copper mediates irreversible, oxidative cross-linking of A β via modified tyrosine residues [25,26]. In the prevention cohort, the radically lower plasma Cp in the first two months of treatment and lower overall average Cp may have had a more profound effect on amyloid pathology. Cp values could not be reduced further in the treatment paradigm due to weight loss. In any event, our findings suggest that systemic copper-lowering strategies may achieve anti-amyloid effects in human subjects if initiated early in the disease process.

It may be important to emphasize that the copper-lowering strategy employed here is distinct from the copper redistribution strategy exemplified by clioquinol and PBT2 [27]. Copper lowering with TM has the advantage that it can be actively titrated during treatment, but it has the disadvantage of potentially causing copper deficiency. Although excessive copper depletion is associated with hematologic and neurologic toxicity, the titration of TM dose to a target plasma Cp level and body weights minimized toxicity in these experiments. Brain activity of copper-dependent enzymes was not impaired by treatment, and sensorimotor function as measured by rotarod and open field testing was also unaffected by TM treatment.

The evidence that the anti-amyloid effect was beneficial for the neurologic function of the mice is less compelling. Since neuronal loss is absent in this model, and since even synaptic density is not consistently altered, we rely on behavioral testing to test for neuroprotectant effects. The spatial memory of the mice was not improved in either experiment, despite a fairly robust effect

on insoluble A β in the prevention paradigm. This may suggest that systemic copper lowering is not sufficient to initiate a neuroprotective response. This is consistent with the concept that redistribution of metals rather than systemic metal depletion is necessary to initiate a neuroprotective response.

On the other hand, it may be relevant that the spatial memory test is a measure of hippocampal function, while the amyloid lowering effect in the prevention experiment was greater in the cerebral cortex than in the hippocampus. An anti-amyloid effect in the cerebral cortex would be expected to be silent on the Morris water maze test of spatial memory, as we observed, but might have significant cognitive benefits in human subjects. Future studies would measure cognition-specific cortical function using the novel object recognition test. The negative spatial memory results therefore may not be a sound basis for evaluating the functional significance of this copper-lowering strategy.

If a copper lowering strategy is to be applied in human subjects, safety will be the limiting factor. Since the amyloid-lowering effect was more robust in the “prevention” experiment compared to the “treatment” experiment, copper lowering appears to be most promising as a preventive strategy. Acceptable levels of toxicity are especially low in prevention studies initiated in asymptomatic individuals. TM is probably too potent to use in frail elderly subjects. Nevertheless, other copper lowering strategies are available, and in light of our promising findings, worth pursuing.

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Fig 1.

TM was titrated to plasma ceruloplasmin. Plasma ceruloplasmin (Cp) activity in both wild type and Tg2576 mice treated with either vehicle (veh) or tetrathiomolybdate (TM) in (a) the prevention and (b) the treatment cohorts. Cp activity was measured in units of activity per ml by the oxidase method from 6–8 randomly chosen mice from each treatment group at each timepoint. Data (means $\pm$ SE) is expressed as % activity compared to vehicle treatment.

Fig 2.

TM lowered brain copper. Frontal cortex copper concentrations expressed as ng copper per mg wet tissue weight in (a) the prevention and (b) treatment cohorts. wt veh = wild type mice receiving vehicle (n=15 prevention, n=10 treatment); wt TM = wild type mice receiving TM (n=13 prevention, n=12 treatment); Tg veh = Tg2576 mice receiving vehicle (n=13 prevention, n=6 treatment); Tg TM = Tg2576 mice receiving TM (n=13 prevention, n=7 treatment). All results are mean $\pm$ SE. Bars with an * differ at $p < 0.05$ compared to corresponding vehicle-treated animals.

Fig 3.

Copper and Molybdenum levels increased in the livers of TM-treated animals. (a) Liver copper and (b) molybdenum in a subset of animals from the prevention cohort (n=6). Data is expressed as ng metal per mg wet tissue weight. Molybdenum was non-detectable (ND) in the livers of vehicle-treated animals. All results are mean $\pm$ SE. Bars with an * differ at $p < 0.05$ compared to corresponding vehicle-treated animals.

Fig 4.

TM treatment does not alter copper-dependent enzyme activity. Results are mean enzyme activity $\pm$ SE in brain homogenates from the prevention cohort for (a) cytochrome c oxidase (cytoco) and (b) superoxide dismutase (SOD). wt veh = wild type mice receiving vehicle (n=8–10); wt TM = wild type mice receiving TM (n=7); Tg veh = Tg2576 mice receiving vehicle (n=7); Tg TM = Tg2576 mice receiving TM (n=6–8).

Fig 5.

TM had no effect on rotarod or open field performance. (a) Rotorod performance for the prevention cohort. Data points represent mean duration time on the rod $\pm$ SE. (b) Open field performance for the prevention cohort. Data points reflect mean distance moved $\pm$ SE. wt veh = wild type mice receiving vehicle (n=16); wt TM = wild type mice receiving TM (n=13); Tg veh = Tg2576 mice receiving vehicle (n=14); Tg TM = Tg2576 mice receiving TM (n=14).

Fig 6.

TM reduced insoluble brain amyloid in the prevention cohort. (a) Soluble A β 1–40 (b) insoluble A β 1–40 and (c) insoluble A β 1–42 as determined by ELISA. (d) Quantification of histological A β burden in the cortex and hippocampus. (e) Representative images of coronal sections from the right hemisphere from vehicle and TM-treated Tg2576 mice. Micrographs were taken from a similar region of the brain, with cerebral cortex occupying the upper half and hippocampus the lower half of each figure. Tg veh = Tg2576 mice receiving vehicle (n=14); Tg TM = Tg2576 mice receiving TM (n=14). Graph results are mean $\pm$ SE, bars with an * differ at $p < 0.05$.

Fig 7.

TM had no effect on insoluble brain amyloid in the treatment cohort. (a) soluble Aβ1-40 (b) soluble Aβ1-42 (c) insoluble Aβ1-40 and (d) insoluble Aβ1-42 as determined by ELISA. (e) Histological Aβ burden in the cortex and hippocampus. Tg veh = Tg2576 mice receiving vehicle (n=6); Tg TM = Tg2576 mice receiving tetrathiomolybdate (n=7). Graph results are mean ± SE, bars with an * differ at p < 0.05.

Fig 8.

TM treatment did not affect APP processing. (a) Relative expression of beta secretase (BACE), presenilin (PSEN) and murine and human A β PP (mAbPP, hAbPP) normalized to expression of GAPDH, determined by Quantitative real time PCR. (b) Representative western blot for A β PP and tubulin and (c) quantification of A β PP protein expression normalized to GAPDH for the prevention cohort.

Fig 9.

TM treatment did not improve spatial memory in the Morris water maze test. (a) Mean $\pm$ SE duration of time required for mice to find a visible platform in the prevention cohort. (b) Mean $\pm$ SE escape latencies per each four-trial block of hidden platform for the prevention cohort. Spatial memory was significantly impaired in both vehicle and TM-treated Tg2576 mice after trial 6 ($p < 0.05$). (c) Probe test of spatial memory retention 24 h after the last hidden platform trial. Mean $\pm$ SE measures of percent dwell in the target quadrant for the prevention cohort. wt veh = wild type mice receiving vehicle ($n = 16$ prevention, $n = 10$ treatment); wt TM = wild type mice receiving TM ($n = 14$); Tg veh = Tg2576 mice receiving vehicle ($n = 13$); Tg TM = Tg2576 mice receiving TM ($n = 14$ prevention).