

Triethylenetetramine Pharmacology and Its Clinical Applications

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Abstract

Triethylenetetramine (TETA), a Cu^{II}-selective chelator, is commonly used for the treatment of Wilson's disease. Recently, it has been shown that TETA can be used in the treatment of cancer because it possesses telomerase inhibiting and anti-angiogenesis properties. Although TETA has been used in the treatment of Wilson's disease for decades, a comprehensive review on TETA pharmacology does not exist. TETA is poorly absorbed with a bioavailability of 8 to 30%. It is widely distributed in tissues with relatively high concentrations measured in liver, heart, and kidney. It is mainly metabolized via acetylation, and two major acetylated metabolites exist in human serum and urine. It is mainly excreted in urine as the unchanged parent drug and two acetylated metabolites. It has a relatively short half-life (2 to 4 hours) in humans. The most recent discoveries in TETA pharmacology show that the major pharmacokinetic parameters are not associated with the acetylation phenotype of *N*-acetyltransferase 2, the traditionally regarded drug acetylation enzyme, and the TETA-metabolizing enzyme is actually spermidine/spermine acetyltransferase. This review also covers the current preclinical and clinical application of TETA. A much needed overview and up-to-date information on TETA pharmacology is provided for clinicians or cancer researchers who intend to embark on cancer clinical trials using TETA or its close structural analogs. *Mol Cancer Ther*; 9(9); 2458–67. ©2010 AACR.

Introduction

Triethylenetetramine (TETA), a Cu^{II}-selective chelator and an orphan drug, is commonly used for the treatment of Wilson's disease (1). Recently, its potential uses in cancer chemotherapy and other diseases are under investigation.

Wilson's disease is an autosomal recessive genetic disorder, manifested by copper accumulation in the tissues of patients (2). Illness presents as neurologic or psychiatric symptoms and liver disease, resulting in the death of patients, and was considered an incurable disease until the 1950s. Treatments of this disease using orphan drugs were developed in the 1950s by John Walshe (3). Currently, common treatments for Wilson's disease either reduce copper absorption, by using zinc acetate, or remove the excess copper from the body using chelators such as penicillamine and TETA (4).

Recently, it was shown that TETA could ameliorate left ventricular hypertrophy in humans and rats with diabetes (5–7). It has also been suggested that TETA can

be used in the treatment of cancer because it is a telomerase inhibitor (8), and has anti-angiogenesis properties (9–11), on the basis of preclinical investigations. In addition, a recent report showed that TETA treatment could overcome cisplatin resistance in human ovarian cancer cell culture via inhibition of superoxide dismutase 1/Cu/Zn superoxide dismutase (12). Another recent report showed that TETA could induce apoptosis in murine fibrosarcoma cells by activation of the p38 mitogen-activated protein kinase (MAPK) pathway (13). However, no clinical trial or trial plan using TETA to treat cancer has been reported in the literature. Because TETA is an orphan drug and has been used in the clinic for decades, it can be tested readily in clinical cancer chemotherapy. However, in order to take advantage of the possible benefits of TETA in clinical cancer treatment, a thorough understanding of TETA pharmacology is crucial.

Although TETA has been used in the treatment of the Wilson's disease for decades, relatively few reports on TETA pharmacology in patients with Wilson's disease can be found in the literature (1, 14), and no comprehensive review of TETA pharmacology exists to date. This overview examines pharmacologic aspects of TETA and its current clinical applications, thus providing valuable information to research scientists or clinicians who are interested in using TETA as a treatment for cancer or other diseases. It also reveals the gaps in TETA pharmacology that need to be addressed, despite its decades of clinical use in patients with Wilson's disease.

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Chemistry and Detection

TETA is a structure analog of linear polyamine compounds spermidine and spermine (see Fig. 1). It was first made in Berlin, Germany in 1861 and was made as a dihydrochloride salt in 1896 (15). Its chelation activity was studied at Cambridge University in 1925 (15). Cu^{II} prefers nitrogen to oxygen as a ligand, and because TETA has four nitrogen groups, it fits the square-planar geometry in which Cu^{II} is most stable (Fig. 1). Therefore, it binds Cu^{II} very tightly, having a dissociation constant from Cu^{II} of 10^{-15} mol/L at pH 7.0 (15).

TETA is mainly used in the clinic in the form of dihydrochloride salt (trientine; refs. 1, 16); although, a TETA disuccinate form has recently been developed as well (17). Trientine dissolves in aqueous solutions and presents as a free-based TETA. The detection of TETA in aqueous solutions has proven to be difficult because TETA has a very polar structure, does not elute efficiently from conventional high performance liquid chromatography (HPLC) columns, and possesses little absorbance at

accessible UV detection wavelengths. One solution, inspired by aqueous polyamine analytic methods (18), is to use fluorescence-labeling reagents to derivatize TETA and detect its derivatives by using a fluorimetric detector. A number of fluorescence-labeling reagents have been tried, including *m*-toluoyl chloride, fluorescamine, dansyl chloride, *O*-phthalaldehyde, 4-(1-pyrene)butyric acid *N*-hydroxysuccinimide ester, and 9-fluorenylmethylchlorofomate (19–25). However, fluorimetric methods are associated with challenges, such as whether the analyte is fully or partially labeled, and whether detected peaks are separated from other known or unknown metabolites, polyamines, and their metabolites. Only one of the above methods (23) addressed those concerns. An HPLC-conductivity detection method has also been developed (26), but its detection limit is relatively high, rendering poor sensitivity to the method. Recently, a nonderivatized method using liquid chromatography-mass spectrometry (LC-MS) has been developed to detect TETA and its two major metabolites simultaneously in aqueous solutions (27), providing more sensitive detection and analytic power. With the availability of the LC-MS-MS technology, a method with higher sensitivity and accuracy could be developed to study TETA and its metabolites in human samples, which will certainly facilitate future pharmacologic studies of TETA.

Pharmacokinetics

Summary of pharmacokinetic parameters in preclinical and clinical situations

Pharmacokinetic parameters in preclinical studies are summarized in Table 1. Most preclinical studies were done on rats (20, 28–34); only one study on dogs (21) and one study on rabbits (22) have been reported. Early studies used ^{14}C -labeled TETA hydrochloride salts. With the development of new analytic methods (HPLC and LC-MS methods), later studies used a typical TETA dihydrochloride salt.

Pharmacokinetic parameters in clinical studies are summarized in Table 2. Clinical results were obtained from healthy volunteers (22, 23, 35–40), patients presenting with Wilson's disease (20, 31, 41), and type 2 diabetes patients (38). Detailed pharmacokinetic parameters listed in Tables 1 and 2 are discussed in relation to absorption, distribution, metabolism, and excretion in the next four sections.

Absorption in animals

Results obtained from rat and dog studies show that TETA has a relatively slow absorption and apparently incomplete intestinal absorption. The T_{max} for rats, dogs, and rabbits after oral TETA administration is 0.5 to 2 hours (Table 1), indicating an overall slow gut absorption. The intestinal absorption rate in normal male Wistar rats has been reported to be 42% in the jejunum and 22.5% in the ileum using an *in situ* loop method (30). In

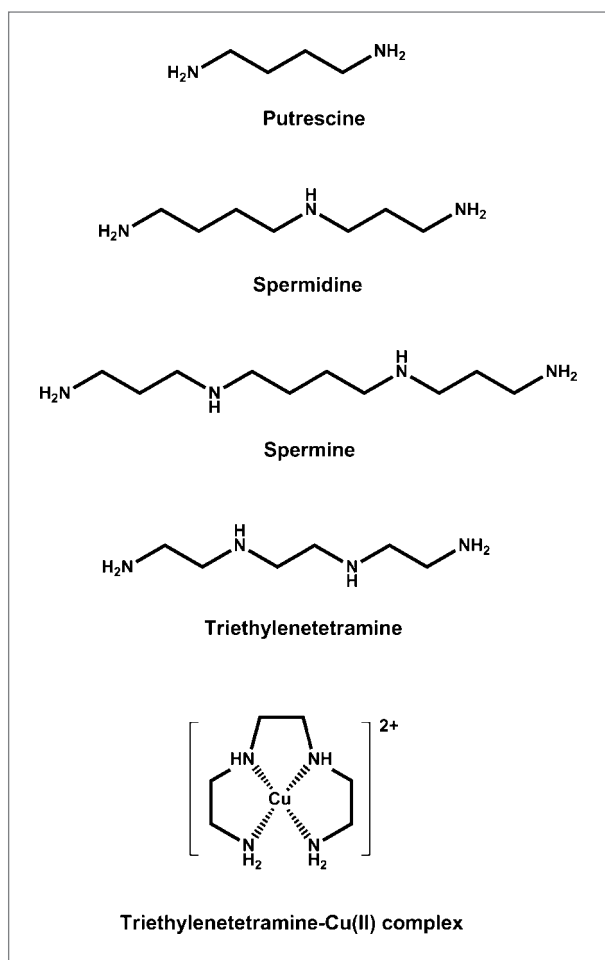


Figure 1. Structures of polyamines, TETA, and TETA- Cu^{II} complex.

Table 1. Summary of pharmacokinetic parameters of TETA in preclinical studies in animals

| Reference number | Species | Dose (mg/kg) | Percent Bioavailability | Percent of dose urinary recovery | | AUC (mg × h/L) | T _{1/2} (hours) | T _{max} (hours) | C _{max} (mg/L) |
|------------------|---------|--------------|-------------------------|----------------------------------|----------------------|----------------|--------------------------|--------------------------|-------------------------|
| | | | | Unchanged parent | Parent + Metabolites | | | | |
| (28) | Rat | 26 (fasted) | 6.0–18.0 | — | — | — | 0.5 | — | — |
| (30) | Rat | 25 (fasted) | 6.6 | 3.5 | 35.7 | — | 2.0–3.0 | 2.0 | 8.0 |
| | | 25 (feed) | 2.3 | — | — | — | 1.5 | 0.5–1.0 | 3.0 |
| (20) | Rat | 25 (fasted) | 5.6–16.4 | — | — | — | — | — | — |
| (29) | Rat | 25 (fasted) | — | — | 5.0–8.0 | — | — | — | — |
| (34) | Rat | 25 (fasted) | — | — | 79.1 | — | 1.3–1.9 | 1.0 | 2.1–26.5 |
| (33) | Rat | 25 (fasted) | — | 2.6 | 39.5 | — | — | — | — |
| (32) | Rat | 25 (fasted) | 25.5 | — | — | 6.6 | 1.0 | 0.5 | 3.8 |
| | | 25 (feed) | 14.0 | — | — | 3.6 | 1.6 | 0.5 | 1.3 |
| (31) | Rat | 25 (fasted) | 13.8 | — | — | 24.0 | ~1.5 | 0.5 | ~11.0 |
| (21) | Dog | 50 (fasted) | — | — | — | 25.6 | 1.6 | 0.9 | 11.2 |
| | | 125 (fasted) | — | — | — | 157.9 | 1.5 | 1.0 | 57.3 |
| | | 300 (fasted) | — | — | — | 428.5 | 1.9 | 1.1 | 114.2 |
| (22) | Rabbit | 150 (fasted) | — | — | — | — | ~2.0 | 2 | 16.3 |

Long-Evans Cinnamon (LEC) rats, the model organism for Wilson's disease, the jejunum absorption rate has been reported to be approximately 46%, and without statistical significance when compared with data derived from Wistar rats (29). In Sprague Dawley rats, the extent of absorption after oral TETA administration has been reported to be 44.3% (34). *In vitro* studies have been carried out to determine the uptake characteristics of TETA by rat intestinal brush-border membrane vesicles (31, 42, 43). The mechanism of absorption is similar to those of physiologic polyamines, such as spermine and spermidine, with respect to excessive accumulation in vesicles, pH dependency, temperature dependency, and the ineffectiveness of K⁺ diffusion potential. The initial uptake of TETA has a K_m value of 1.1 mmol/L, which is larger than that observed for spermine and spermidine. The uptake rate of TETA can be inhibited in a dose-dependent manner by spermine and spermidine.

The bioavailability range of oral trientine in fasted rats was first reported at 6 to 18% (28). Later reports provided similar results. One study reported a bioavailability of 2.31% in nonfasted rats and 6.56% in fasted rats (30). A second report showed bioavailability in three fasted rats at 5.6%, 5.7%, and 16.4%, respectively (20). A third report provided a bioavailability of 14.0% in nonfasted rats and 25.5% in fasted rats (32). A fourth report determined that the bioavailability in fasted rats was 13.78% (31). Overall, the bioavailability of oral TETA administration is relatively low in rats, and food intake seems to reduce it further.

Absorption in humans

In humans, the absorption rate in the gut after oral trientine administration is also relatively slow. The T_{max}

values from various studies occur between 0.8 to 4 hours (Table 2), indicating overall slow absorption. There seems to be a large variance of C_{max} (0.4 to 20 mg/L) among different individuals with Wilson's disease who had been dosed with 600 mg trientine in one early study (20). However, the C_{max} variance seems to be less profound in healthy volunteers on the same dose, that is, 0.80 ± 0.33 mg/L (mean ± SD) in one recent study (40), and 0.69 ± 0.41 mg/L in another recent report (39). Food intake inhibits absorption, as shown by reduced C_{max} and decreased area under the curve (AUC) in one healthy volunteer (22). The parameter of bioavailability has not been measured in humans.

Distribution in animals

TETA is widely distributed into various tissues in rats, either in the form of unchanged parent compound or biotransformed metabolite(s). The earliest study done by Gibbs and Walshe using ¹⁴C radio-labeled TETA-4HCl showed that liver, kidney, and muscle had higher TETA concentrations than those quantified in plasma (28). A later study using ¹⁴C radio-labeled trientine showed that TETA could be found in most rat tissues, including cerebrum, cerebellum, hypophysis, eyeball, harderian gland, thyroid, submaxillary gland, lymphatic gland, thymus, heart, lung, liver, kidney, adrenal, spleen, pancreas, fat, brown fat, muscle, skin, bone marrow, testis, epididymis, prostate gland, stomach, small intestine, and large intestine (34). However, concentrations in liver and kidney seemed to be much higher than those in plasma, and plasma concentrations were higher than those observed for other tissues. Apart from liver and kidney, other tissues

did not accumulate significant amounts of TETA after oral administration. In the analyses, it was observed that both the parent compound and metabolite(s) exist in all tissues (33). A later report confirmed such findings, showing that concentration ratios of liver/plasma and kidney/plasma were greater than 1, whereas brain, lung, spleen, and white fat have ratios lower than 1 (31).

It is proposed that TETA shares a common transport mechanism with polyamines in intestinal uptake. It is likely that TETA is also transported across biological membrane into mammalian cells by the same transporter for polyamines. The transporter of polyamines has been identified as glypican-1 (44). Inside cells, polyamines are further transported into mitochondria, where polyamine concentrations can reach millimolar level, electrophoretically by a specific polyamine uniporter (45). It is therefore not surprising that TETA is widely distributed in the body and can be accumulated in the tissues.

Distribution in humans

No data are available for tissue distribution in humans. Because the bioavailability has not been established in humans, the volume of distribution cannot be calculated from previously published studies. However, a recent study reported that the central and

peripheral volumes of distribution were 393 L and 252 L, respectively (39). These values indicate that TETA is widely distributed in the human body, where accumulation in certain tissues is likely to happen.

Metabolism in animals

TETA is extensively metabolized in rats. *In vitro* experiments have shown that about 50% of TETA was eliminated from the S9 liver fraction system after 2 hours of incubation (29). One *in vivo* study in rats showed that after oral administration of trientine, only 3.1% of the dose was found in the 24-hour urine collection as the unchanged parent compound, whereas metabolites accounted for 32.6% of the oral dose (30). Another *in vivo* study reported that 2.6% of the dose was recovered from 24-hour urine collection as the unchanged parent compound, and 11% metabolites (33). The existence of acetylated metabolites in rats was first proposed, then established by Gibbs and Walshe (28). To date, two acetylated metabolites, *N*₁-acetyltriethylenetetramine (MAT; refs. 36, 37) and *N*₁,*N*₁₀-diacetyltriethylenetetramine (DAT; refs. 27, 38), have been identified. TETA metabolite levels in rat tissues have been investigated in two studies. In one study, after oral administration of trientine, the plasma AUC_{0 to 6 h} of the metabolite MAT has been reported

Table 2. Summary of pharmacokinetic parameters of TETA in clinical studies'

| Reference number | Subject | No. of individuals | Dose (mg/d) | Percent of dose urinary recovery | | AUC (mg × h/L) | T _{1/2} (hours) | T _{max} (hours) | C _{max} (mg/L) |
|------------------|---------|--------------------|----------------|----------------------------------|----------------------|----------------|--------------------------|--------------------------|-------------------------|
| | | | | Unchanged parent | Parent + Metabolites | | | | |
| (20) | WDP | 8 | ~600 | — | — | — | 2.0–4.0 | 2.0–30 | 0.5–10.0 |
| (35) | HV | 2 | ~2,000 | 2.3 | — | — | — | — | — |
| (36) | HV | 1 | 1,250 | 4.1 | 11.8 | — | — | — | — |
| (41) | WDP | 10 | ~500–1,800 | — | — | — | 1.3–3.5 | 1.6–3.5 | 0.8–14.0 |
| | | 12 | 750–2,500 | 2.4 | 23.3 | — | — | — | — |
| (31) | WDP | 8 | ~1,750 | 1.6 | 10.4 | 6.4 | 2.0–4.0 | 1.0–3.0 | 1.2–4.2 |
| (37) | HV | 3 | 1,000 | 1.0 | 10.0 | — | — | — | — |
| (22) | HV | 1 | 1,500 (fasted) | — | — | — | 4.0 | 2.0 | 3.8 |
| | | | 1,500 (feed) | — | — | — | 8.0 | 4.0 | 1.5 |
| (38) | HV | 6 | 300–2,400 | 0.7 | 3.2 | — | — | — | — |
| | | | DBP | 7 | 300–2,400 | 0.6 | 9.2 | — | — |
| (39) | HV | 8 | 200* | — | — | 1.23 | 5.1 | 0.8 | 0.4 |
| | | | 600* | — | — | 2.9 | 5.4 | 1.1 | 0.7 |
| | | | 1,200* | — | — | 10.0 | 10.4 | 0.8 | 1.6 |
| | | | 3,600* | — | — | 20.0 | 14.2 | 0.9 | 5.6 |
| (40) | HV | 24 | 600 | — | — | 3.0 | 2.5 | 1.8 | 0.8 |
| | | | 600* | — | — | 4.2 | 3.3 | 2.0 | 1.0 |

NOTE: Unless specifically labeled, all data are from fasted first dose.

Abbreviations: WDP, Wilson's disease patient; HV, healthy volunteers; DBP, diabetic patient.

*After repeated dosing.

to be higher than that of unchanged TETA in rats (31). Both the same report and another early report (33) showed that MAT existed in rat tissues at similar levels observed for the unchanged parent compound.

Metabolism in humans

TETA is extensively metabolized in humans, as a number of metabolites have been found in urine other than the unchanged parent compound. Two major TETA metabolites have been identified from human urine, both of which are acetylation products of TETA. MAT was first identified in 1993 (36), and further studied in 1997 (37). DAT was first identified in 2007 (27), and further studied together with MAT in both healthy volunteers and patients affected with diabetes (38, 40).

Most of the absorbed TETA dose is excreted as either unchanged parent compound or metabolites in urine, as bile excretion seems to be minimal, shown in one study in which less than 0.8% of intravenous-administered TETA was excreted via bile excretion (34). The majority of the urinary excreted TETA is in the form of metabolites, MAT, and DAT. The recovery of unchanged parent compound in urine ranges from 0.71 to 4.10% of the administered dose in healthy volunteers, and from 0.64 to 2.40% in patients with Wilson's disease or diabetes (Table 2). Metabolite(s) recovery ranges from 2.50 to 9.00% in healthy volunteers; and, from 8.56 to 27.1% in patients with diabetes or Wilson's disease (Table 2). It is suggested that patients with diabetes have a higher rate of TETA metabolism than healthy volunteers (38). Whether other disease states, such as Wilson's disease or cancer, have the same effect on TETA metabolism as diabetes has not been established, but further investigation is warranted. It is worth noticing that cancer-derived cytokines may repress the activity of drug-metabolizing enzymes, especially cytochrome P450 enzymes (46).

The enzyme responsible for TETA metabolism has yet to be formally identified. Because two major metabolites have been identified as acetylation products of TETA, it is natural to suggest that the major drug acetylation enzyme, *N*-acetyltransferase (NAT2), is responsible for TETA's acetylation. However, a recent study showed that there is no correlation between the NAT2 acetylation phenotype and metabolic rate of TETA (40). This lack of correlation suggests another enzyme may be responsible for TETA's metabolism. A current study conducted by our laboratory shows that spermidine/spermine acetyltransferase (SSAT) is the enzyme responsible for the formation of two of the TETA acetylation metabolites.³ Given the fact that TETA is a structural analog of spermidine and spermine, it is not surprising that SSAT is the enzyme that metabolizes TETA in humans. SSAT may also be responsible for the metabolism of many other polyamine analogs,

³ Lu J. Unpublished data.

such as diethylspermine and diethylnorspermine, which are currently in clinical trials for the treatment of cancer (47).

Excretion and/or elimination in animals

Most of the absorbed TETA that is excreted via urine as bile and lung excretions seems to be minimal in animal studies. One study found that after oral trientine administration to rats, 0.69% of the dose was found in expired air and 0.86% of the dose was excreted via bile (34). The urinary excreted TETA is mainly in the form of acetylated metabolites, whereas the unchanged parent compound represents a smaller percentage of the dose (Table 1). The renal clearance of TETA in rat is about 30% higher than creatinine clearance, which indicates that TETA is actively excreted from the renal tubule into urine (48). It has been identified that the Na⁺/spermine antiporter in the rat renal tubular brush-border membrane is responsible for active excretion of spermine, TETA, and any other straight-chain polyamine compound with more than four amino groups (49). TETA metabolites MAT and DAT, are also straight-chain structures, and with four amino groups, they should be able to be actively excreted in kidney as well. Therefore, it is not surprising that a large number of metabolites are found in rat urine.

Diseases that compromise kidney function in rats seem to affect urinary excretion of TETA. One early study reported that LEC rats, a rat model of Wilson's disease, had significantly lower urinary TETA excretion than that in normal Wistar rats. This lower rate was due to the impairment of kidney function in LEC rats (29).

The plasma elimination half-lives ($T_{1/2}$) of TETA in rat, dog, and rabbit are between 0.5 to 2 hours (Table 1), which suggests that TETA is quickly removed from the blood.

Excretion and/or elimination in humans

Most of the urinary excreted TETA is in the form of the unchanged parent compound and two acetylated metabolites, MAT and DAT (38). Patients affected with diabetes excrete more metabolites in urine than healthy volunteers (38). It has been reported that urinary excretion of spermine is elevated in patients with certain types of cancer (50, 51). The implication of these facts for TETA excretion is unknown because the mechanism of TETA urinary excretion in humans has yet to be established.

Urinary concentrations of Cu, Fe, and Zn all increased in parallel with TETA excretion (6, 37). Trientine administration has also been shown to increase the fecal excretion of Cu in Wilson's disease patients (52).

The plasma elimination $T_{1/2}$ of TETA in healthy volunteers and Wilson's disease patients ranges from 1.3 to 4 hours (Table 2), indicating fast elimination of the parent compound. The $T_{1/2}$ increases to approximately 3 to 5 hours after repeated dosing at 200 and 600 mg/d (39, 40), and reaches 10 to 14 hours after repeated dosing

at 1,200 and 3,600 mg/d (39). The acetylation phenotype of NAT2 does not have any effect on the TETA elimination $T_{1/2}$ (40). TETA's metabolites, MAT and DAT, have much longer $T_{1/2}$ than that of TETA itself. The MAT $T_{1/2}$ is around 5.3 hours and DAT $T_{1/2}$ is around 10.8 hours. After 7 days of repeated dosing at 600 mg/d, MAT $T_{1/2}$ reached 9 hours and DAT $T_{1/2}$ reached 14 hours (40).

Drug-drug interactions

It has been shown in a rat study that diuretics, such as acetazolamide and furosemide, can increase the urinary TETA excretion (53). In contrast, drugs that are the substrate of the H^+ /organic cation antiporter or aminoglycoside antibiotics do not interact with TETA in terms of excretion (53). Diuretics are the drugs that change the concentration of sodium ions in renal proximal tubules. The increase in the luminal concentration of sodium ions accelerates the Na^+ /spermine antiporter, which is responsible for the active excretion of TETA into urine. No drug interaction information in humans is currently available. Only a few drugs are metabolized via the acetylation route, and even fewer drugs are possibly metabolized via the SSAT route. This observation implicates that there may be few drug-drug interactions, because metabolizing enzyme activation or competition is unlikely between TETA and most of other drugs.

Adverse Drug Reactions, Toxicity, and Safety

Clinical experience with TETA (in the form of trientine) has been predominantly limited to patients with Wilson's

disease (1, 16, 54–67). The reported side effects are summarized in Table 3. In addition, a recent clinical trial also reported a safety profile of trientine in healthy volunteers with various NAT2 phenotypes (40). In general, trientine has a relatively safe clinical profile, and reported side effects seem to be minor.

For teratogenicity, no studies exist with trientine in pregnant women unaffected by Wilson's disease. One series report and two case reports can be found on the use of trientine during pregnancy in Wilson's disease patients. The series report (68) reviewed seven patients with Wilson's disease treated by trientine, who had been followed during 11 pregnancies. Eight of these resulted in the delivery of normal infants. One premature birth occurred at 31 weeks, which was later shown to have a chromosomal abnormality. There was one therapeutic termination for noncompliance of the patient with the drug, and one miscarriage associated with a contraceptive coil. The eight normal infants were reported as progressing satisfactorily, and had been studied for periods varying from 3 months to 9 years. All mothers were also reported as doing well. The normal-for-age ceruloplasmin values found in the cord blood indicates no significant Cu depletion in the fetuses as a result of trientine treatment. In the first case of the two separated case reports, trientine was only used in the last 1 to 2 weeks prior to delivery, and delivery of a normal baby at 34 weeks by cesarean section was recorded (69). In the second case, trientine had been used for 8 years prior to conception. Trientine was continued throughout the pregnancy, and a normal child was delivered via cesarean section at 42 weeks (70). Current usage of trientine during pregnancy for

Table 3. Reported clinical side effects of TETA in Wilson's disease patients

| No. of patients | Trientine dose (mg/d) | Treatment duration | Reported side effects | Additional comments | Reference |
|-----------------|-----------------------|---------------------|--|--|-----------------------|
| 20 | 1,200–2,400 | >1 year | Fe deficiency in most patients | Resolved with Fe replacement | Walshe (1) |
| 7 | 500–2,000 | 6 weeks to 16 years | One had mild thrombocytopenia at 1,750 mg/d | Resolved on 1,000 mg/d | Dubois et al. (55) |
| 4 | 1,000–3,000 | 2 months | One had mild and transient numbness in the lips | — | Saito et al. (56) |
| 1 | 1,000–2,250 | 1.5 years | Development of microcytic sideroblastic anemia at 6 months | Resolved on 1,000 mg/d | Condamine et al. (59) |
| 19 | 1,000–1,800 | 8.5 years | One had Fe deficiency, one had low serum Zn, two had colitis on 1,500 mg/d | Fe and Zn deficiency resolved with supplements, one colitis resolved with lower dose, and one discontinued | Dahlman et al. (60) |
| 1 | 2,400 | >4 years | Development of microcytic sideroblastic anemia at 2 years | Resolved on 1,200 mg/d | Perry et al. (61) |
| 23 | 1,000 | 3 years | One had anemia | — | Brewer et al. (65) |
| 10 | 500–1,000 | 5 years | One had mild liver toxicity | Switched to zinc acetate | Arnon et al. (66) |
| 13 | 600–2,400 | >6 years | One has allergic rash | Trientine discontinued | Taylor et al. (16) |

Wilson's disease is only recommended when the potential benefit outweighs the potential risk to the fetus.

Pharmacodynamics

Mechanism of action in Wilson's disease

TETA is a Cu^{II} -selective chelator, which aids the systemic elimination of divalent Cu from the human body by forming a stable complex that is readily excreted from the kidney (Fig. 1; refs. 6, 15, 71). TETA not only increases urinary Cu excretion, but also decreases intestinal copper absorption by 80% (57). TETA and its metabolite, MAT, are both capable of binding divalent Cu, Fe, and Zn. However, the chelating activity of MAT is significantly lower than that of TETA (37). The urinary levels of copper increase in parallel with the amount of TETA excretion in healthy volunteers (37, 38), but increase in parallel with the sum of TETA and MAT in diabetic patients (38). The removal of excessive Cu in Wilson's disease patients is regarded as its mechanism of action for treating this disease.

Mechanism of action in cancer

A few mechanisms have been proposed to be the possible mode of action of TETA on cancer cells. A few reports have shown that TETA could be a telomerase inhibitor (8, 72–74). By inhibiting telomerase, TETA might have the selective inhibitory effect or cytotoxicity on tumor growth, because telomerase is an essential factor in cellular immortalization and tumorigenesis, which are expressed in more than 85% of all human cancers. It has been suggested that TETA inhibits telomerase because it is a ligand for G-quadruplex, and stabilizes both intra- and intermolecular G-quadruplexes (72, 75).

Another mechanism of TETA action in cancer is thought to be anti-angiogenesis. Copper plays a key role in angiogenesis (76). Chelation of copper by TETA suppresses several angiogenic mediators, including vascular endothelial growth factor-1 (VEGF-1), fibroblast growth factor-1 (FGF-1), interleukin-1 (IL-1), IL-6, IL-8, and nuclear factor kappa-light-chain-enhancer of activated B cells (NF- κ B; refs. 9–11, 77–80). As a result, TETA exhibits anti-angiogenic effects in tumor cells.

Promotion of apoptosis is another proposed mechanism. One recent report suggested that TETA could induce apoptosis in murine fibrosarcoma cells via the activation of the p38 MAPK pathway (13). Another report suggested that prolonged copper depletion via TETA chelation might induce expression of antioxidants and trigger apoptosis in neuroblastoma cells (81).

TETA can also be used to overcome cisplatin resistance in ovarian cancer cells by decreasing the overexpressed Cu/Zn superoxide dismutase (12). Because it is well established that TETA can decrease the overexpressed Cu/Zn superoxide dismutase in human diseases, combination therapy using cisplatin and TETA could be a possible clinical entry point for TETA chemotherapy in cancer.

Currently, there is no systemic study to investigate the anticancer mechanisms of TETA. In order to better understand TETA's anticancer effects, more systemically designed studies are needed to show the hierarchy of TETA action in cancer cells, which will certainly guide or benefit future clinical application.

Mechanism of action in other clinical applications

Recently, TETA has been used in clinical trials for the treatment of diabetic heart failure. It has been shown that there is a hyperglycemia-driven pathogenic abnormality of copper homeostasis in type 2 diabetic patients (5). TETA treatment reduces left ventricular hypertrophy in patients (7). It also improves left ventricular function (5, 82), restores damaged aortic and left ventricular structures (5, 83), and improves cardiac antioxidant defense (83–85) in rat models of diabetes. However, the exact target(s) and mechanism of actions of TETA in diabetic heart failure are still under investigation.

Apart from diabetic heart failure, TETA is effective in treating other diabetic complications. One report has shown that TETA was effective in diabetic nephropathy in a rat model through normalizing renal fibrosis and pathogenic transforming growth factor- β (TGF- β) activation (85). Two other reports produced data that showed that TETA suppressed carbonyl stress and reduced inflammation in the lenses of diabetic rats. Hence, TETA could be used to assist the treatment of diabetic retinopathy (86, 87).

Current Clinical Applications and Therapeutic Implications

TETA is currently used as the second line treatment, in the form of trientine, for Wilson's disease, mainly for those patients with penicillamine allergy or intolerance (14, 88, 89). It is more commonly used in children with Wilson's disease (16, 66). The common dosing schedule is twice a day at 600 mg/d, which is determined mainly by its plasma $T_{1/2}$. TETA is also used in other metal intoxications. For example, one case reported that trientine was effective for the treatment of manganese intoxication in one patient with acquired hepatocerebral degeneration (90).

Another clinical use of TETA is in diabetic complications. TETA has been used in clinical trials to treat diabetic heart failure, and has been shown to be effective in patients diagnosed with type 2 diabetes presenting cardiac complications (5, 7). Several preclinical animal studies have been carried out using TETA to treat diabetic nephropathy and retinopathy, and the results show that TETA is effective in ameliorating those complications in diabetic animal models (85–87).

For the treatment of cancer using TETA, a number of preclinical *in vivo* and *in vitro* studies have been carried out. However, only one clinical application of TETA in cancer has been reported. In that report, TETA was used to reduce liver copper content in patients with hepatocellular carcinoma (HCC) after percutaneous ethanol

injection or radiofrequency ablation (91). It showed that TETA could reduce copper content in the liver tissue, which also could be beneficial in the treatment of HCC because an increased level of copper has been identified in association with HCC development.

In preclinical studies, TETA has been shown to effectively inhibit the growth of various tumors or tumor cells, including neuroblastoma, HCC, HeLa cells, colorectal carcinoma, breast cancer cells (MCF-7), fibrosarcoma, and glioma, through the mechanisms of anti-angiogenesis, telomerase inhibition, and apoptosis (8–11, 13, 72–75, 77, 80, 81). In another study, TETA exhibited the ability to overcome cisplatin resistance in human ovarian cancer cells via inhibition of the activity of Cu/Zn superoxide dismutase (12). On the basis of the same mechanism, TETA has been shown to be effective in the treatment of familial amyotrophic lateral sclerosis, which is a copper-mediated oxidative toxicity, in a mouse model (92). According to one *in vitro* study (93), it has been suggested that TETA may be effective in treating Alzheimer's disease.

Copper seems to be an essential element for angiogenesis in cancer cells. One potent copper chelator, tetrathiomolybdate, is currently in clinical trials for cancer (94–96). On the other hand, polyamine seems to play an important role in cancer nutrition, and a number of polyamine analogs are in clinical trials as well (47, 97). As a copper-specific chelator and polyamine analog with a relatively safe clinical profile and distinctive metabolic pathway from common cancer drugs, TETA is a good candidate for cancer chemotherapy or combination therapy.

Summary

TETA is an established orphan drug with promising new clinical applications and implications. TETA may be a promising anticancer agent with the potential to enter clinical trials very soon. It is also likely to be used

in combination with cancer chemotherapy. As a result, information about its pharmacology is greatly needed. This review provides a rough overview of the known pharmacology of TETA on the basis of available information. Even though TETA has been used in clinical situations for decades, information about its pharmacology is still limited. For example, many pharmacologic aspects of TETA have not been fully investigated, such as the exact mechanism of absorption in humans, the impact of zinc coadministration on its absorption, which food ingredients inhibit its absorption, how is it released from cells, how it passes the blood-brain barrier, the implication of kidney impairment on its excretion, and its full-scale mechanism of action in cancer treatment. Recent investigation on its metabolism suggests that it could be an ideal candidate for combination chemotherapy, as it is metabolized via a unique SSAT pathway that is unlikely to interact and/or interfere with the metabolism of normal anticancer drugs. More pharmacologic information about TETA is still needed, especially in population groups with diseases, such as cancer and diabetes patients. A thorough understanding of TETA pharmacology is essential for it to be adopted in cancer chemotherapy.

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No potential conflicts of interest were disclosed.

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