Triethylenetetramine Pharmacology and Its Clinical Applications

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Abbreviations: DAT, \(N_1,N_{10}\)-diacetyltriethylenetetramine; FGF, fibroblast growth factor; HCC, hepatocellular carcinoma; HPLC, high performance liquid chromatography; IL-1, interleukin-1; LC-MS, liquid chromatography-mass spectrometry; LEC rat, Long-Evans Cinnamon rat; MAT, \(N_1\)-acetyltriethylenetetramine; NAT2, \(N\)-acetyltransferase 2; NF-\(\kappa\)B, nuclear factor kappa-light-chain-enhancer of activated B cells; SSAT, spermidine/spermine \(N\)-acetyltransferase; TETA, triethylenetetramine; TGF-\(\beta\), transforming growth factor-\(\beta\); trientine, triethylenetetramine dihydrochloride; VEGF, vascular endothelial growth factor.

Notes:

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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 demonstrated that TETA can be used in the treatment of cancer because it possesses telomerase inhibiting and antiangiogenesis properties. Although TETA has been used in the treatment of Wilson’s disease for decades, there is no comprehensive review about TETA pharmacology. TETA is poorly absorbed with a bioavailability of 8-30%. 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 (2-4 h) 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 currently pre-clinical and clinical application of TETA. It will provide a much-needed overview and up-to-date information on TETA pharmacology for clinicians or cancer researchers who intend to embark on cancer clinical trials using TETA or its close structural analogs.
Introduction

Triethylenetetramine (TETA), a Cu\textsuperscript{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 tissues of patients (2). Illness presents as neurological or psychiatric symptoms and liver disease, resulting in 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 demonstrated 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 antiangiogenesis properties (9-11) based on pre-clinical 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 MARK pathway (13). However, no clinical trial or trial plan using TETA to treat cancer has been reported in the literature. Since TETA is an orphan drug and has been used in clinic for decades, it can be tested readily in clinical cancer chemotherapy. However, in order to take the 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, there are relatively few reports on TETA pharmacology in patients with Wilson’s disease in the literature (1, 14) and no comprehensive review of TETA pharmacology exists to date. This overview examines pharmacological aspects of TETA and its current clinical applications, which will provide 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.

Chemistry and Detection

TETA is a structure analog of linear polyamine compounds spermidine and spermine (see Figure 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). CuII prefers nitrogen to oxygen as a ligand, and because TETA has four nitrogen groups, it fits the square-planar geometry in which CuII is most stable (Figure 1). Therefore, it binds CuII very tightly, having a dissociation constant from CuII of 10^-15M at pH 7.0 (15).

TETA is mainly used in the clinic in the form of dihydrochloride salt (trientine) (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 it possesses little absorbance at accessible UV detection wavelengths. One solution, inspired by aqueous polyamine analytical 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 \textit{m}-toluoyl chloride, fluorescamine, dansyl chloride, \textit{O}-phthalaldehyde, 4-(1-pyrene)butyric acid \textit{N}-hydroxysuccinimide ester, and 9-flouorenlymethylchlorofomate (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 and 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 non-derivatized 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 analytical power. With the availability of the LC-MS/MS technology, method with higher sensitivity and accuracy could be developed to study TETA and its metabolites in human samples, which will certainly facilitate future pharmacological studies of TETA.

**Pharmacokinetics**

**Summary of Pharmacokinetic Parameters in Pre-clinical and Clinical Situations**

Pharmacokinetic parameters in pre-clinical studies are summarized in Table 1. Most pre-clinical studies were performed on rats (20, 28-34); only one study on dogs (21) and one study on rabbits (22) have been reported. Early studies used \textsuperscript{14}C-labeled TETA hydrochloride salts. With the development of new analytical 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 Wilson’s disease (20, 31, 41), and type 2 diabetes patients (38). Detailed pharmacokinetic parameters listed in Tables 1 and 2 will be discussed in relation to absorption, distribution, metabolism and excretion in next four sections.

**Absorption – Animals**

Results obtained from rat and dog studies show that TETA has a relatively slow absorption and apparently incomplete intestinal absorption. The $T_{\text{max}}$ for rats, dogs and rabbits after oral TETA administration is 0.5-2 h (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 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 physiological 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 mM, 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 6-18% (28). Later reports provided similar results. One study reported a bioavailability of 2.31% in non-fasted 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 non-fasted 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 – Humans

In humans, the absorption rate in the gut after oral trientine administration is also relatively slow. The T\text{max} values from various studies occur between 0.8-4 h (Table 2), indicating overall slow absorption. There seems to be a large variance of C\text{max} (0.4 – 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\text{max} variance seems to be less profound in healthy volunteers on the same dose, i.e. 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\text{max} and decreased AUC in one healthy volunteer (22). The parameter of bioavailability has not been measured in humans.

Distribution – 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 performed by Gibbs and Walshe using \textsuperscript{14}C radio-labeled TETA·4HCl showed that liver, kidney and
muscle had higher TETA concentrations than that quantified in plasma (28). A later study using $^{14}$C radiolabeled 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 appeared to be much higher than that in plasma, and plasma concentrations were higher than that 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, while 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 – Humans**

There is no existing data for tissue distribution in humans. Since the bioavailability has not been established in humans, the volume of distribution cannot be calculated from previous 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 – 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 hr 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-hr urine collection as the unchanged parent compound, while 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-h 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) (36, 37) and $N_1,N_{10}$-diacetyltriethylenetetramine (DAT) (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$_{0-6hr}$ of the metabolite MAT has been reported to be higher than that of unchanged TETA in rats (31). The same report and another early report (33) both showed that MAT existed in rat tissues at similar levels observed for the unchanged parent compound.

**Metabolism – 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 where less than 0.8% of i.v.-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 has not been established, but deserve attention to be further investigated. It is worth noticing that cancer-derived cytokines may repress the activity of drug metabolizing enzymes, especially those cytochrome P450 enzymes (46).

The enzyme responsible for TETA metabolism has yet to be formally identified. Since 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 suggests another enzyme may be responsible to 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.
(unpublished data). 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, such as diethylspermine and diethylnorspermine, which are currently in clinical trials for the treatment of cancer (47).

**Excretion/Elimination – Animals**

Most of the absorbed TETA is excreted via urine as bile and lung excretions appear 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, while 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 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 4 amino groups (49). TETA metabolites MAT and DAT, are also straight-chain structures, and with 4 amino groups, they should be able to be actively excreted in kidney as well. Therefore, it is not surprising that a large amount 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 significant lower urinary TETA excretion than that in normal Wistar rats. This 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 – 2 h (Table 1). This suggests that TETA is quickly removed from the blood.

**Excretion/Elimination – 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 to 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 h to 4 h (Table 2), indicating fast elimination of the parent compound. The T_{1/2} increases to approximately 3 – 5 h after repeated dosing at 200 and 600 mg/day (39, 40), and reaches 10 – 14 h after repeated dosing at 1200 and 3600 mg/day (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 h and DAT T_{1/2} is around 10.8 h. After 7 days of repeated dosing at 600 mg/day, MAT T_{1/2} reached 9 h and DAT T_{1/2} reached 14 h (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). On the other hand, 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 ion 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 implicates that there may be little drug-drug interactions, due to the fact that 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 appear to be minor.

Regarding teratogenicity, there are no studies with trientine in pregnant women unaffected by Wilson’s disease. There is one series report and two case reports on the use of trientine during the 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. There was one premature birth at 31 weeks, which was later shown to have a chromosomal abnormality.
There was one therapeutic termination for non-compliance 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 three months to nine years. All mothers were also reported as doing well. The normal-for-age ceruloplasmin values found in the cord blood indicates that there was 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 1-2 weeks prior to delivery, and delivery of a normal baby at 34 weeks by cesarean section was recorded (69). In the second case, trientine has been used for eight 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 Wilson’s disease is only recommended where 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 (Figure 1) (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 is expressed in over 85% of all human cancers. It is suggested that TETA inhibit telomerase because it is a ligand for G-quadruplex, and stabilizes both intra- and inter-molecular G-quadruplexes (72, 75).

Another mechanism of TETA action in cancer is thought to be antiangiogenesis. Copper plays a key role in angiogenesis (76). Chelation of copper by TETA suppresses several angiogenic mediators, including VEGF-1, FGF-1, IL-1, IL-6, IL-8 and NF-κB (9-11, 77-80). As a result, TETA exhibits antiangiogenic 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 over-expressed Cu/Zn superoxide dismutase (12). Since it is well established that TETA can decrease the over-expressed 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 anti-cancer mechanisms of TETA. In order to better understand TETA’s anti-cancer 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 TGF-β activation (85). Two other reports produced data that demonstrated 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/day, which is determined mainly by its plasma half-life. 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 pre-clinical animal studies have been carried out using TETA to treat diabetic nephropathy and retinopathy, and the results show that TETA is effective for ameliorating those complications in diabetic animal models (85-87).

For the treatment of cancer using TETA, a number of pre-clinical in vivo and in vitro studies have been carried out. However, there is only one clinical application of TETA in cancer that 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 could be also beneficial to the treatment of HCC because increased level of copper has been identified in association with HCC development.

In pre-clinical studies, TETA has been show to be effectively inhibiting 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 antiangiogenesis, 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). Based on 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). There is also a suggestion that TETA may be effective in treating Alzheimer’s disease according to one in vitro study (93).

Copper appears 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 relative 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 cancer chemotherapy. As a result, information about its pharmacology is greatly needed. The review herein provides a rough overview of the known pharmacology of TETA based on available information. Even though TETA has been used in clinical situations for decades, information about its pharmacology is still limited. For example, many pharmacological aspects of TETA have not been fully investigated, such as the exact mechanism of absorption in humans, the impact of zinc co-administration on its absorption, which food ingredients inhibit it absorption, how is it released from cells, how it passes 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 treatment.
chemistry as it is metabolized via a unique SSAT pathway that is unlikely to interact/interfere with the metabolism of normal anticancer drugs. More pharmacological information about TETA is still needed, especially in population group with diseases, such as cancer and diabetes patients. Thorough understanding of TETA pharmacology is essential for it to be adopted in cancer chemotherapy.
References:


Table 1. Summary of pharmacokinetic parameters of triethylenetetramine (TETA) in pre-clinical studies in animals.

<table>
<thead>
<tr>
<th>Reference Number</th>
<th>Species</th>
<th>Dose (mg/kg)</th>
<th>Bioavailability (%)</th>
<th>Urinary Recovery (% of dose)</th>
<th>AUC (mg·h/L)</th>
<th>Half-life (h)</th>
<th>Tmax (h)</th>
<th>Cmax (mg/L)</th>
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<td></td>
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<td></td>
<td>Unchanged parent</td>
<td>Parent + metabolites</td>
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<td></td>
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<tr>
<td>(28) Rat</td>
<td>26 (fasted)</td>
<td>6.0-18.0</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>0.5</td>
<td>-</td>
<td>-</td>
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<tr>
<td>(30) Rat</td>
<td>25 (fasted)</td>
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<td>3.5</td>
<td>35.7</td>
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<td>25 (feed)</td>
<td>2.3</td>
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<td></td>
<td></td>
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<td>0.5-1.0</td>
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<tr>
<td>(20) Rat</td>
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<tr>
<td>(29) Rat</td>
<td>25 (fasted)</td>
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<td>-</td>
<td>5.0-8.0</td>
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<td>-</td>
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<td>(34) Rat</td>
<td>25 (fasted)</td>
<td>-</td>
<td>-</td>
<td>79.1</td>
<td>1.3-1.9</td>
<td>1.0</td>
<td>2.1-26.5</td>
<td></td>
</tr>
<tr>
<td>(33) Rat</td>
<td>25 (fasted)</td>
<td>-</td>
<td>2.6</td>
<td>39.5</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>(32) Rat</td>
<td>25 (fasted)</td>
<td>25.5</td>
<td>-</td>
<td>-</td>
<td>6.6</td>
<td>1.0</td>
<td>0.5</td>
<td>3.8</td>
</tr>
<tr>
<td></td>
<td>25 (feed)</td>
<td>14.0</td>
<td></td>
<td></td>
<td>3.6</td>
<td>1.6</td>
<td>0.5</td>
<td>1.3</td>
</tr>
<tr>
<td>(31) Rat</td>
<td>25 (fasted)</td>
<td>13.8</td>
<td>-</td>
<td>-</td>
<td>24.0</td>
<td>~1.5</td>
<td>0.5</td>
<td>~11.0</td>
</tr>
<tr>
<td>(21) Dog</td>
<td>50 (fasted)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>25.6</td>
<td>1.6</td>
<td>0.9</td>
<td>11.2</td>
</tr>
<tr>
<td></td>
<td>125 (fasted)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>157.9</td>
<td>1.5</td>
<td>1.0</td>
<td>57.3</td>
</tr>
<tr>
<td></td>
<td>300 (fasted)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>428.5</td>
<td>1.9</td>
<td>1.1</td>
<td>114.2</td>
</tr>
<tr>
<td>(22) Rabbit</td>
<td>150 (fasted)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>~2.0</td>
<td>2</td>
<td></td>
<td>16.3</td>
</tr>
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</table>
Table 2. Summary of pharmacokinetic parameters of triethylenetetramine (TETA) in clinical studies. Unless specifically labelled, all data are from fasted first dose. WDP, Wilson’s disease patient; HV, healthy volunteers; DBP, diabetic patient.

<table>
<thead>
<tr>
<th>Reference Number</th>
<th>Subject</th>
<th>Number</th>
<th>Dose (mg/day)</th>
<th>Urinary Recovery (% of dose)</th>
<th>AUC (mg·h/L)</th>
<th>Half-life (h)</th>
<th>T_max (h)</th>
<th>C_max (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Unchanged parent</td>
<td>Parent + Metabolites</td>
<td></td>
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<tr>
<td>(20) WDP</td>
<td>8</td>
<td>~600</td>
<td>-</td>
<td>-</td>
<td>2.0-4.0</td>
<td>2.0-30</td>
<td>0.5-10.0</td>
<td></td>
</tr>
<tr>
<td>(35) HV</td>
<td>2</td>
<td>~2000</td>
<td>2.3</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>(36) HV</td>
<td>1</td>
<td>1250</td>
<td>4.1</td>
<td>11.8</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>(41) WDP</td>
<td>10</td>
<td>~500-1800</td>
<td>-</td>
<td>-</td>
<td>1.3-3.5</td>
<td>1.6-3.5</td>
<td>0.8-14.0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>750-2500</td>
<td>2.4</td>
<td>23.3</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>(31) WDP</td>
<td>8</td>
<td>~1750</td>
<td>1.6</td>
<td>10.4</td>
<td>6.4</td>
<td>2.0-4.0</td>
<td>1.0-3.0</td>
<td>1.2-4.2</td>
</tr>
<tr>
<td>(37) HV</td>
<td>3</td>
<td>1000</td>
<td>1.0</td>
<td>10.0</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>(22) HV</td>
<td>1</td>
<td>1500 (fasted)</td>
<td>-</td>
<td>-</td>
<td>4.0</td>
<td>2.0</td>
<td>3.8</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>1500 (feed)</td>
<td>-</td>
<td>-</td>
<td>8.0</td>
<td>4.0</td>
<td>1.5</td>
<td></td>
</tr>
<tr>
<td>(38) HV</td>
<td>6</td>
<td>300-2400</td>
<td>0.7</td>
<td>3.2</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td></td>
<td>DBP</td>
<td>7</td>
<td>300-2400</td>
<td>0.6</td>
<td>9.2</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>(39) HV</td>
<td>8</td>
<td>200*</td>
<td>-</td>
<td>-</td>
<td>1.23</td>
<td>5.1</td>
<td>0.8</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>8</td>
<td>600*</td>
<td>-</td>
<td>2.9</td>
<td>5.4</td>
<td>1.1</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>8</td>
<td>1200*</td>
<td>-</td>
<td>10.0</td>
<td>10.4</td>
<td>0.8</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>8</td>
<td>3600*</td>
<td>-</td>
<td>20.0</td>
<td>14.2</td>
<td>0.9</td>
<td></td>
</tr>
<tr>
<td>(40) HV</td>
<td>24</td>
<td>600</td>
<td>-</td>
<td>-</td>
<td>3.0</td>
<td>2.5</td>
<td>1.8</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>600*</td>
<td>-</td>
<td>-</td>
<td>4.2</td>
<td>3.3</td>
<td>2.0</td>
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</table>

* After repeated dosing.
Table 3. Reported clinical side effects of TETA in Wilson’s disease patients

<table>
<thead>
<tr>
<th>Patients (number)</th>
<th>Trientine dose (mg/day)</th>
<th>Treatment duration</th>
<th>Reported side effects</th>
<th>Additional comments</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>20</td>
<td>1200-2400</td>
<td>&gt; 1 year</td>
<td>Fe deficiency in most patients</td>
<td>Resolved with Fe replacement</td>
<td>Walshe, 1982 (1)</td>
</tr>
<tr>
<td>7</td>
<td>500-2000</td>
<td>6 weeks – 16 years</td>
<td>1 had mild thrombocytopenia at 1750 mg/day</td>
<td>Resolved on 1000 mg/day</td>
<td>Dubois et al., 1990 (55)</td>
</tr>
<tr>
<td>4</td>
<td>1000-3000</td>
<td>2 months</td>
<td>1 had mild and transient numbness in the lips</td>
<td>-</td>
<td>Saito et al., 1991 (56)</td>
</tr>
<tr>
<td>1</td>
<td>1000-2250</td>
<td>1.5 years</td>
<td>Development of microcytic sideroblastic anemia at 6 month</td>
<td>Resolved on 1000 mg/day</td>
<td>Condamine et al., 1993 (59)</td>
</tr>
<tr>
<td>19</td>
<td>1000-1800</td>
<td>8.5 years</td>
<td>1 had Fe deficiency 1 had low serum Zn 2 had colitis on 1500 mg/day</td>
<td>Fe and Zn deficiency resolved with supplements 1 colitis resolved with lower dose and 1 discontinued</td>
<td>Dahlman et al., 1995 (60)</td>
</tr>
<tr>
<td>1</td>
<td>2400</td>
<td>&gt; 4 years</td>
<td>Development of microcytic sideroblastic anemia at 2 year</td>
<td>Resolved on 1200 mg/day</td>
<td>Perry et al., 1996 (61)</td>
</tr>
<tr>
<td>23</td>
<td>1000</td>
<td>3 years</td>
<td>1 had anemia</td>
<td>-</td>
<td>Brewer et al., 2006 (65)</td>
</tr>
<tr>
<td>10</td>
<td>500-1000</td>
<td>5 years</td>
<td>1 had mild liver toxicity</td>
<td>Switched to Zinc acetate</td>
<td>Arnon et al., 2007 (66)</td>
</tr>
<tr>
<td>13</td>
<td>600-2400</td>
<td>&gt; 6 years</td>
<td>1 has allergic rash</td>
<td>Trientine discontinued</td>
<td>Taylor et al., 2009 (16)</td>
</tr>
</tbody>
</table>
Figure Legend:

Figure 1. Structures of polyamines, triethylenetetramine, and triethylenetetramine-Cu(II) complex.
Figure 1

Putrescine

Spermidine

Spermine

Triethylenetetramine

Triethylenetetramine-Cu(II) complex
# Molecular Cancer Therapeutics

## Triethyleneetetramine Pharmacology and Its Clinical Applications

Jun Lu

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<tr>
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<td>Author manuscripts have been peer reviewed and accepted for publication but have not yet been edited.</td>
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</table>

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