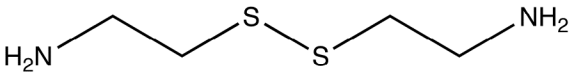
CINAPS Compound Dossier

Cystamine



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National Institute of Neurological Disorders and Stroke

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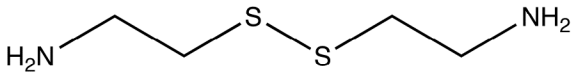
I. Compound Information

Common name: Cystamine

Structure:



Pubchem ID: 2915 **Mol. Formula:** $C_4H_{12}N_2S_2$ **FW:** 152.28

CASRN: 51-85-4 **Polar surface area: 57.08** **logP:** -0.1

IUPAC name: 2-(2-Aminoethyldisulfanyl)ethanamine

Other names: Diaminodiethyldisulfide; Decarboxycystine

Drug class: Transglutaminase inhibitor; antioxidant; caspase-3 activation inhibitor; cysteamine pro-drug

Medicinal chemistry development potential: High

II. Rationale

IIa. Scientific Rationale / Mechanism

Proteinaceous aggregates containing α-synuclein lead to the formation of cytoplasmic inclusions (Lewy bodies) that are implicated in cellular toxicity, neuronal dysfunction and neurodegenerative diseases such as Parkinson's disease, dementia with Lewy bodies, and multiple system atrophy. α-Synuclein is a small protein of 140 amino acids consisting of three molecular domains including an N-terminal lipid-binding amphipathic α-helix, a central amyloid-binding domain fragment representing the non-Aβ component of Alzheimer plaques, and a C-terminal acidic tail. Depending upon its cellular milieu, α-synuclein exists in either a natively unfolded conformation or as an α-helix in the presence of phospholipid vesicles, suggesting a dynamic regulation of its function depending on the local cellular environment. In its unfolded structure, α-synuclein is prone to self-aggregate and to cause the aggregation of other proteins, a property that may underlie its involvement in Lewy body formation and its contribution to the pathogenesis of Parkinson's disease. *In vitro*, α-synuclein is capable of self-aggregating into fibrils in a time-, temperature-, pH-, and concentration-dependent manner. Other factors such as mutations, C-terminal truncation, metal ions, and oxidative stress have also been shown to increase α-synuclein aggregation *in vitro*. Many of these same factors are known to increase α-synuclein aggregation in cellular and animal models of Parkinson's disease. These include pathogenic α-synuclein mutations, oxidative stress, proteasomal impairment, mitochondrial defects, and interaction with other proteins, such as parkin and synphilin-1. Thus, the identification and characterization of mechanisms and triggers for α-synuclein aggregation *in vivo* is critical because such factors are likely to be involved in the pathogenesis of Parkinson's disease and other α-synucleinopathies[1].

Recent studies in several neurodegenerative processes have demonstrated a potential involvement of tissue transglutaminase in their pathogenesis through the catalysis of protein aggregation. Transglutaminases are a family of proteins that catalyze a calcium-dependent acyl transfer reaction between the carboxamide group of a polypeptide-bound glutamine and the amino group of a polypeptide-bound lysine residue that results in cross-linking of proteins *via* (γ glutamyl) lysine bonds. Most importantly, tissue transglutaminase 2 catalyzes the formation of α-synuclein aggregates *in vitro* as well as in cellular models, and (γ glutamyl) lysine bonds have been demonstrated in Lewy bodies in Parkinson's disease and dementia with Lewy bodies[1]. These findings implicate this enzyme in the formation of Lewy bodies by cross-linking α-synuclein and possibly in the pathogenesis of α-synucleinopathies. Furthermore, in Alzheimer's disease, tissue transglutaminase catalyzes the cross-linking of neurofilament, amyloid precursor protein, tau, and non-Aβ component. In addition, tissue transglutaminase activity and expression are elevated in affected areas of Alzheimer's brains compared with normal controls. Studies have also stressed the role of tissue transglutaminase in polyglutamine expansion neurodegenerative disorders, such as Huntington's disease, as polyglutamine residues are excellent targets for this enzyme, and tissue transglutaminase activity and expression also appear elevated in brain regions affected by Huntington's disease [1]. Furthermore, Mastroberadino *et al.* showed that

TGase 2 knockout in transgenic mice expressing exon 1 of huntingtin containing an expanded polyglutamine results in improved life expectancy and amelioration of neurological symptoms.

Cystamine is known to inhibit tissue transglutaminase activity through a disulfide exchange reaction and serves as a competitor for tissue transglutaminase by blocking access of the glutamine residue in substrate proteins to the active site of the enzyme. Moreover, cystamine is able to inhibit tissue transglutaminase-induced α-synuclein aggregation in cellular models. However, treatment with cystamine in cell systems does not appear to resolve preexisting protein aggregate inclusions[1].

Interestingly, in one study the administration of cystamine *in vivo* to transgenic mice expressing exon 1 of huntingtin containing an expanded polyglutamine repeat did not affect the appearance or the frequency of neuronal inclusions, yet still altered the course of their Huntington's-like disease. When treatment began after the appearance of abnormal movements, cystamine extended survival, reduced associated tremor and abnormal movements and ameliorated weight loss. Treatment did not influence the appearance or frequency of neuronal nuclear inclusions. Instead, cystamine treatment increased transcription of one of the two genes shown to be neuroprotective for polyglutamine toxicity in Drosophila, dnaj (also known as HDJ1 and Hsp40 in humans and mice, respectively)[2]. *In vitro*, cystamine increased cellular glutathionine levels, and inhibited the *in situ* activation of caspase-3 by different pro-apoptotic agents in a concentration-dependent manner[3]. This mechanism of action is particularly relevant in the context of Huntington's disease as inhibition of caspases delays the disease progression in mouse models. Cystamine has also been reported to promote secretion of brain-derived neurotrophic factor (BDNF) in brains of Huntington's disease mice to produce a neuroprotective effect, and increase serum levels of BDNF in mouse and primate models of Huntington's disease [4]. Finally, cystamine may be converted to the potent antioxidant cysteamine *in vivo*, suggesting that it may possess therapeutic potential in neurodegenerative disorders through a variety of mechanisms.

There is substantial evidence that free radical scavengers and antioxidants such as cystamine can attenuate the oxidative stress, mitochondrial dysfunction, and associated apoptotic activity *via* caspase activation that are believed to underlie Parkinson's disease and other neurodegenerative disorders. In one study in mice, orally-administered cystamine was able to ameliorate MPTP-induced nigral neuron loss, preserve striatal dopaminergic projections, and improve striatal dopamine and dopamine metabolite levels. Cystamine reduced striatal 8-hydroxy-2'-deoxyguanisine (8-OH2dG) levels and normalized ATP concentrations, consistent with reduced oxidative stress and improved mitochondrial function. In accord with improved mitochondrial function and survival, cystamine also protected against 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-induced elevations in cytochrome c and caspase activities[5].

In most of the studies showing a protective effect of cystamine in chemically-induced neuropathologies and their progression, it is not clear how much cystamine crosses the blood-brain barrier, and once in the brain, how much of the compound is converted to its reduced form, cysteamine. Under normal circumstances the glutathione/glutathione disulfide ratio in the brain *in vivo* is >100, sufficiently high that most cystamine entering the brain, or formed therein, is predicted to be converted to cysteamine by disulfide interchange with glutathione. However, at

the high concentrations used in some preclinical studies, it still remains likely that some of the cystamine was present as the disulfide. This is important since, cystamine, but not cysteamine, is able to inactivate those enzymes present in brain that require an active site cysteine for catalytic activity, including tissue transglutaminases and the apoptotic enzyme caspase 3. However, cysteamine can act as an amine substrate for tissue transglutaminase 2, and the resulting covalent attachment of cysteamine to a glutaminyl residue will prevent cross linking of that residue to a protein lysyl residue, again possibly preventing protein aggregation. Thus, cystamine may have beneficial effects through its inhibition of tissue transglutaminases, caspase 3, and its conversion to cysteamine, as an antioxidant and less destructive substrate for tissue transglutaminases in brain[6].

IIb. Consistency

n/a

III. Efficacy (Animal Models of Parkinson's Disease)

IIIa. Animal Models: Rodent

Cystamine, or its reduced form of active antioxidant/free radical scavenger cysteamine, has demonstrated significant clinical potential in rodent models of neurodegenerative disorders and human neurological disease [5, 7, 8]. In one of the more comprehensive studies in mice, the oral efficacy of cystamine in the MPTP and 6-hydroxydopamine (6-OHDA) neurotoxin models of Parkinson's disease was determined [5]. In the MPTP studies, male wild type mice with the B6CBA background were randomly distributed into one of six treatment groups at 8.5 months of age: untreated (no cystamine and no MPTP), MPTP-treated (no cystamine), 120 mg/kg cystamine+MPTP, 250 mg/kg cystamine+ MPTP, 500 mg/kg cystamine+MPTP, or 750 mg/kg cystamine+MPTP. Cystamine was prepared daily and administered in drinking water, with intake monitored to maintain adequate dosing throughout the experiment. All cystamine groups received cystamine treatment for one week prior to MPTP administration. During the second week, cystamine treatment continued and all MPTP treatment groups received intraperitoneal injections of 20 mg/kg MPTP *b.i.d.*, over five days. Cystamine treatment continued for seven days after the last MPTP treatment with mice euthanized at that time point for biochemical and neuropathological analysis. The results of this study revealed the neuroprotective effects of cystamine treatment, in that treatment with cystamine significantly ameliorated nigral neuronal loss, preserved striatal dopaminergic projections, and improved striatal dopamine and metabolite levels, as compared to MPTP alone. Cystamine also normalized striatal 8-OH2dG levels and ATP concentrations, consistent with reduced oxidative stress and improved mitochondrial function. Finally, cystamine also protected against MPTP-induced mitochondrial loss, as identified by mitochondrial heat shock protein 70 and superoxide dismutase 2, with concomitant reductions in cytochrome c and caspase-3 activities.

The neuroprotective value of cystamine was also confirmed in the 6-hydroxydopamine model. An additional cohort of mice were weighed and randomly placed into one of three cystamine treatment groups at 8.5 months of age: untreated (no cystamine and no 6-OHDA), 6-OHDA-treated (no cystamine), and 750 mg/kg cystamine + 6-OHDA (N = 15/group). Cystamine was administered as noted above. After one week of cystamine treatment, 6-OHDA (2.5 µg) was stereotactically injected into the right striatum (coordinates: 0.5 mm anterior to bregma, – 2.0 mm lateral to the midline, and 3.1 mm ventral) under pentobarbital anesthesia (50 mg/kg, i.p.). A Hamilton syringe was stereotactically inserted and allowed to equilibrate for 30 s followed by injection over 60 s. Dwell time was 30 s. Cystamine administration continued for 1 week after surgery. Mice were then euthanized for neuropathological and biochemical analyses. The results of this study revealed that 6-OHDA injections into the right striatum resulted in a prominent ipsilateral reduction of tyrosine hydroxylase immunoreactivity (TH-IR) within the substantia nigra (SN) along with a significant reduction in the number of TH-positive neurons in the SN pars compacta (SNpc) as compared to non-lesioned mice. Using the optimal dose of cystamine (750 mg/kg), there was a marked improvement in TH-positive neurons in the SNpc. Consistent with these findings, dopamine transporter-immunoreactive (DAT-IR) terminals in the striatum,

measured using densitometric analyses, were markedly reduced by 76% after 6-OHDA administration and rescued by cystamine treatment (96% of control). There were significantly elevated levels of 8-OH2dG in the striatum of 6-OHDA-treated mice compared to non-lesioned control mice that were normalized by cystamine administration (750 mg/kg), consistent with the MPTP findings. Together these findings show cystamine's therapeutic benefit to reduce neuronal loss through attenuation of oxidative stress and mitochondrial dysfunction, providing the rationale for human clinical trials in patients with Parkinson's disease [5].

IIIb. Animal Models: Non-human Primates

No Parkinson's-specific studies of cyst(e)amine in non-human primates have been reported to date.

IV. Efficacy (Clinical and Epidemiological Evidence)

IVa. Clinical Studies

Cysteamine is an orphan medication (Cystagon® Mylan Corp.), which is approved for the treatment of pediatric nephropathic cystinosis [9]. When started early in life, cysteamine forestalls the need for renal transplantation, allows normal growth and development, and prevents secondary hypothyroidism. Nausea and vomiting are the most common side effects of this treatment [7]. It is also of interest for its therapeutic potential in the treatment of ocular cystinosis, non-alcoholic steatohepatitis, neuronal ceroid lipofuscinosis, Huntington's disease, and other neuropathologies involving pathogenic protein aggregation, oxidative stress and mitochondrial dysfunction .

The maximum tolerable dose (MTD) and side effects of cysteamine in people with Huntington's disease was recently determined in a single-center open-label study (efficacy was not a clinical endpoint in this study). Cysteamine was started at a dose of 10 mg/kg per day, divided into four doses, and increased by 10 mg/kg per day weekly until the development of intolerable side effects or a maximum dose of 70 mg/kg per day. Of the 9 subjects, 1 had an MTD of 10 mg/kg per day, 1 had an MTD of 20 mg/kg per day, the maximum dose was 30 mg/kg per day for 2, 40 mg/kg per day for 2, and 50 mg/kg per day for 3. The dose found tolerable by 8 of the subjects was 20 mg/kg per day. All had a noticeable hydrogen sulfide odor at doses of 40 mg/kg per day or higher, but nausea and motoric impairment were the dose-limiting side effects. The investigation concluded that, at a dose of 20 mg/kg per day, cysteamine was tolerable in people with Huntington's disease [7].

IVb. Epidemiological Evidence

Immunohistochemical studies on postmortem brain tissue have confirmed the presence of transglutaminase-catalyzed (γ-glutamyl)lysine cross-links in the halo of Lewy bodies in Parkinson's disease and dementia with Lewy bodies, colocalizing with α-synuclein. These findings, taken together, suggest that tissue transglutaminase activity leads to α-synuclein aggregation to form Lewy bodies and perhaps contributes to neurodegeneration. Thus, postmortem studies in human brain suggests that targeting tissue transglutaminases in the brain with cystamine, cysteamine, or more selective transglutaminase inhibitors holds some therapeutic promise[1].

V. Relevance to Other Neurodegenerative Diseases

Several commonalities have been identified in age-related cognitive decline and the etiology of Huntington's, Parkinson's, Alzheimer's, and other neuropathic CNS diseases[10]. These include oxidative stress, mitochondrial dysfunction, protein misfolding, crosslinking and aggregation, and apoptosis. Due to the potential for common underlying pathologies or mechanisms for their progression, it appears that cyst(e)amine has clinical relevance in many of these disease states that can be attributed to its potent antioxidant, free radical scavenging, and caspase and transglutaminase inhibiting activities. Indeed, cystamine has been shown to be neuroprotective in animal models of Huntington's [4] and Parkinson's [8] diseases. However, it should be noted that when infused intracerbroventricularly in rats, cysteamine accelerated the ageing-related accumulation of peroxidase-positive (iron-rich) cytoplasmic inclusions in subcortical (hippocampal) astroglia and induces their appearance in primary neuroglial cultures (see adverse effects). In contrast to the gliopathic changes, no evidence of neuronal or myelin damage was observed in the cysteamine-exposed rats. The cysteamine-treated animals exhibited significant impairment in spatial learning as determined using a three-panel runway task. The working memory deficits were more robust at the end of the drug washout period than immediately following cessation of the cysteamine infusion. Thus, the cysteamine-related memory deficits are of long duration and are not due to any acute neuroactive properties of the drug itself[11].

The Borrell-Pages *et al.* study of cystamine in laboratory animals showed that treatment with the disulfide increases levels of the heat shock DnaJ-containing protein 1b (HSJ1b) that are low in Huntington's disease patients[4]. The studies demonstrated that cystamine positively regulates brain derived neurotrophic factor (BDNF) release not only by increasing HSJ1 transcripts but also by inhibiting tissue transglutaminases. It was also demonstrated that cysteamine is neuroprotective in mouse models of Huntington's disease by enhancing BDNF levels in brain. Indeed, BDNF blood levels are low in mouse and non-human primate models and can be increased by injection of cysteamine. Thus, these studies also supported a potential new treatment for Huntington's disease using the FDA-approved reduced form of cystamine, cysteamine.

Further evidence supporting the use of cystamine as a neuroprotective agent for Huntington's disease pathology is provided by PET imaging studies of cerebral glucose metabolism with [^{18}F]FDG (2-deoxy-2-[^{18}F]fluoro-d-glucose) and striatal dopamine D2 receptor function with [^{11}C]raclopride in R6/2 transgenic Huntington mice[12]. In these studies, the control mice revealed exponentially decreasing glucose utilization in the striatum, cortex and cerebellum as a function of age starting at 58 days. The 50 mg/kg cystamine dose provided significant protection only for the striatum and only minor protection was obtained using lower doses. Striatal binding potential of [^{11}C]raclopride was 1.059+/-0.030 in the control mice, and enhanced in the cystamine treated animals in a dose dependent manner up to 1.245+/-0.063 using the 100 mg/kg dose. Histological analysis confirmed cystamine induced neuroprotection of striatal and cortical neurons and Nissl staining revealed that formation of cellular inclusions was reversed in a dose dependent manner.

VI. Pharmacokinetics

VIa. General ADME

The reduction of cystamine by thiols is a very facile reaction (~103/M/s), and it is believed that cystamine is rapidly reduced by thiol-bearing compounds in plasma. This reduction would be accompanied by the concomitant oxidation of the thiol to a mixed disulfide and the release of cysteamine. The resulting mixed disulfide would then be reduced by a second thiol species to release the remaining cysteamine. The reduction of the RS-cysteamine mixed disulfide could be mediated by plasma GSH, cysteine or albumin. Cysteamine is also short-lived in plasma, in accord with the low reducing potential of plasma, which has only ~10 μM GSH versus the mM amounts found in most cells. Although values of plasma cysteamine of up to 40 μM have been reported in subjects receiving cysteamine for the treatment of cystinosis [13], the samples in these studies were reduced before analysis and therefore reflect the total plasma content of cysteamine rather than the concentration of reduced cysteamine per se. Cysteamine is thought to rapidly reduce plasma cystine to cysteine while being oxidized to cysteine-cysteamine mixed disulfide. Cysteine is used for GSH and protein synthesis, and cysteine-cysteamine mixed disulfide resembles lysine and enters cells *via* amino acid transporters. Cellular GSH would then reduce the cysteine-cysteamine mixed disulfide to cysteine and cysteamine. While cysteine levels were increased, no appreciable amounts of cyst(e)amine were detected in the brains of a transgenic mouse model of Huntington's disease (YAC128 mice) or their wild-type littermates treated daily for 2 weeks with cystamine (225 mg/kg) in their drinking water. Cysteamine could have been oxidized to hypotaurine or taurine through the actions of cysteamine dioxygenase and hypotaurine dehydrogenase, however, the research team was also unable to detect hypotaurine in the brains of cystamine-treated YAC128 mice (and their littermates) and taurine levels were unchanged. These observations suggest that oxidation to hypotaurine and taurine is probably not a major pathway for the catabolism of cysteamine in the brain[14].

The lack of detectable cysteamine in the brains of animals treated with cystamine suggests a rapid utilization of this aminothiol by pathways other than those leading to hypotaurine or taurine. One possible biochemical reaction involves interaction with tissue transglutaminases. It has been demonstrated that these enzymes are suitable targets for cysteamine, leading to the formation of a γ-glutamylcysteamine moiety, and thereby inhibiting the cross linking of proteins/peptides *via* (γ glutamyl) lysine bonds. This alteration would result in an increase in the thiol content of the modified protein and thereby offer greater antioxidant protection in the immediate vicinity of the peptide. Free γ-glutamylcysteamine is also a potent antioxidant. Thus, the formation of γ-glutamylcysteamine may significantly augment the cellular antioxidant defenses[14].

As noted above, pharmacokinetic studies of cystamine or cysteamine are complicated by the rapidity of redox reactions, and care should be used in interpreting the results of studies as to whether the samples were reduced prior to analysis. In one study by Belldina *et al.*, 2003 (where plasma samples were reduced with sodium borohydride prior to analysis), after oral administration to pediatric cystinotic patients, without renal transplant, cysteamine bitartrate exhibited a Tmax of 1.4 h, indicative of a moderate rate of absorption and substantially longer

than the 0.75-h Tmax reported after administration of either cysteamine HCl or phosphocysteamine[13]. Using doses of cysteamine bitartrate one-quarter (0.05 mmol kg−1) those of cysteamine HCl or phosphocysteamine (0.2 mmol kg−1), the Cmax achieved in the current study (36 μm) was approximately three-quarters those reported previously (approximately 50 μm). Thus, it appears that cysteamine bitartrate formulation results in a slower time to peak concentrations and a slightly lower peak concentration.

The low level of cysteamine in mouse brain is consistent with the low levels of radioactivity in mouse brain detected autoradiographically after systemic administration of [35S]cysteamine [15].

VIb. CNS Penetration

Despite the demonstration that cyst(e)amine treatment is beneficial in numerous *in vitro* and *in vivo* animal models of Huntington's disease, Parkinson's disease, and other neuropathic disorders, the metabolism and distribution of cyst(e)amine metabolites in the CNS is still in need of further characterization. In recent studies it was shown that cystamine and "free" cysteamine are undetectable (≤ 0.2 nmol/100 mg protein) in the brains of 3-month-old transgenic (YAC128) mice (or their wildtype littermates) treated daily for 2 weeks with cystamine (225 mg/kg) in their drinking water. No significant changes were observed in brain glutathione and taurine but significant increases were observed in brain cysteine. Nor was cystamine or cysteamine detected in the plasma of YAC128 mice treated daily with cystamine between the ages of 4 and 12 or 7 and 12 months. These findings suggest that cystamine is not directly involved in mitigating Huntington's disease, but that increased brain cysteine or uncharacterized sulfur metabolites may be responsible. Cystamine or cysteamine might also be rapidly reduced and crosslinked to tissue transglutaminases to form a γ-glutamylcysteamine moiety, and thereby inhibiting the cross linking of proteins/peptides *via* (γ glutamyl) lysine bond[14]. Further studies are required to investigate the distribution of cyst(e)amine and its metabolites in the CNS and gain a further understanding of its mechanism of action in neupropathic diseases.

VIc. Calculated log([brain]/[blood]) (Clark Model):

-0.72

VII. Safety, Tolerability, and Drug Interaction Potential

VIIa. Safety and Tolerability

In general, cysteamine is known to be relatively well-tolerated. Indeed, cysteamine has been used as a radiation-protective agent, has been an approved treatment for nephropathic cystinosis for several years, and in May of 2008, the US FDA granted cysteamine (or, mercaptamine bitartrate) orphan drug status for the treatment of Huntington's disease. Adverse reactions or intolerance leading to cessation of the use of cysteamine bitartrate for cystinosis occurred in 8% of patients in US Studies. Common side effects include nausea, vomiting, loss of appetite, diarrhea, fever, drowsiness, rash, and unpleasant breath odor. In some instances, severe skin rashes can develop, such as erythema multiforme bullosa or toxic epidermal necrolysis. CNS symptoms such as seizures, lethargy, somnolence, depression and encephalopathy have been associated with cysteamine. Cysteamine has occasionally been associated with reversible leucopenia and abnormal liver function.

Based upon information derived from administration of cysteamine bitartrate in pediatric patients, cysteamine appears to be relatively tolerable, although side effects and adverse events can be quite severe .

Cysteamine was not mutagenic in the Ames test, produced a negative response in an in-vitro sister chromatid exchange assay in human lymphocytes, but a positive response in a similar assay in hamster ovarian cells. It does not appear to have been tested for its carcinogenic potential in long-term animal studies.

Reproductive toxicity testing was conducted in male and female rats, and cysteamine was found to have no effect on fertility and reproductive at an oral dose of 75 mg/kg/day (450 mg/m2/day, which is 0.4 times the recommended human dose based on surface area). However, at an oral dose of 375 mg/kg/day (2,250 mg/m2/day, 1.7 times the recommended human dose based on body surface area), it reduced the fertility of the adult rats and the survival of their offspring.

It remains to be determined if cysteamine can cause fetal harm when administered to a pregnant woman or can affect reproduction capacity, nor does it appear to be known if cysteamine is excreted in human milk.

A single oral dose of cysteamine at 660 mg/kg was lethal to rats. Symptoms of acute toxicity were reduction of motor activity and generalized hemorrhage in gastrointestinal tract and kidneys.

In one dose range finding and maximum tolerated dose study in humans with Huntington's disease, it was shown that people with Huntington's disease tolerate cysteamine at a dose comparable to that used for nephropathic cystinosis. Interestingly, the dose-limiting side effects were motoric disturbances (trouble with walking, running, and balance), as well as the more anticipated nausea, weight loss, and halitosis. This was a relatively small study ($n < 10$ subjects); and, problems with walking, running, and coordination were not reported in the earlier study of cysteamine in Huntington's disease or in the literature on the use of cysteamine in the treatment

of nephropathic cystinosis. However, high doses of cysteamine given to rats accelerates the formation of senescent glial changes and bas been associated with impaired locomotion and water-maze performance (Justino *et al.*, 1997). In mice, doses from 50 to 250 mg/kg per day, given subcutaneously, are associated with motor activation (head tremor, body shaking, and increased defecation). In other studies, subcutaneous administration of lower doses of cysteamine improved motor learning in mice with no changes in locomotor ability[7].

A final concern with the safety of long-term cyst(e)amine administration involves its non-selectivity at tissue transglutaminases. A variety of tissue transglutaminases exist and are widely distributed throughout the human body. Furthermore, transglutaminases are involved in cell proliferation and differentiation, extracellular matrix organization, blood coagulation, skin maturation, and suppression of sperm immunogenicity in the female genital tract. The more relevant transglutaminase for neuropathic disorders appears to be tissue transglutaminase 2, which is thought to be involved in apoptosis, cell adhesion and signal transduction. Because tissue transglutaminases are widely distributed in the human body, are under complex gene expression control, and serve as important factors in a number of biological processes, the risk of adverse effects from their inhibition needs to be clearly recognized[6]. A selective inhibitor of transglutaminase 2 might represent the best candidate for therapeutic benefit and minimal side effects. Towards this end researchers have been examining the three dimensional structures of the active sites of tissue transglutaminases and designing irreversible mechanism-based suicide transglutaminase inhibitors. It has been suggested that X-ray crystallographic analysis of transglutaminase 2 with or without GDP, Ca2+ or the inhibitors present might prove most useful in the design of a more potent and selective second generation of transglutaminase 2 inhibitors[6, 16, 17].

The safety and efficacy of cystamine for use in treatment of neurological disease is less well defined. Cystamine dihydrochloride has an oral LD50 value (acute) of 896 mg/kg in the Rat and 874 mg/kg in the mouse. Acute health effects of ingestion include gastrointestinal (digestive) tract irritation with nausea, vomiting and diarrhea. Cystamine may also affect behavior (somnolence, convulsions) and respiratory system (respiratory depression). Chronic exposure health effects include the potential for reproductive toxicity based on animal data.

VIIb. Drug Interaction Potential

Cysteamine decreases levels of somatostatin and has been shown to affect behavior, growth hormone and insulin levels in laboratory animals. It may also chemically react with nitroaromatic compounds and other reactive functional groups on therapeutic agents.

VIII. Bibliography

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