Bioscience Reports (2018) **38** BSR20180691
https://doi.org/10.1042/BSR20180691

PORTLAND PRESS

Perspective

Cystamine and cysteamine as inhibitors of transglutaminase activity *in vivo*

Thomas M. Jeitner*, John T. Pinto and Arthur J.L. Cooper

Department of Biochemistry and Molecular Biology, New York Medical College, Valhalla, NY, USA

Correspondence: Thomas M. Jeitner (tmj4001@med.cornell.edu)

Cystamine is commonly used as a transglutaminase inhibitor. This disulphide undergoes reduction *in vivo* to the aminothiol compound, cysteamine. Thus, the mechanism by which cystamine inhibits transglutaminase activity *in vivo* could be due to either cystamine or cysteamine, which depends on the local redox environment. Cystamine inactivates transglutaminases by promoting the oxidation of two vicinal cysteine residues on the enzyme to an allosteric disulphide, whereas cysteamine acts as a competitive inhibitor for transamidation reactions catalyzed by this enzyme. The latter mechanism is likely to result in the formation of a unique biomarker, *N*-(γ-glutamyl)cysteamine that could serve to indicate how cyst(e)amine acts to inhibit transglutaminases inside cells and the body.

Introduction

Cystamine is a symmetric organodisulphide commonly used as an inhibitor of transglutaminases. This disulphide is also reduced to cysteamine within the body. Cystamine and cysteamine both inhibit transglutaminases but by different mechanisms. Therefore, the purpose of this discussion is to highlight the redox behavior of cystamine and cysteamine *in vivo* and the mechanisms by which cystamine and cysteamine inhibit the activity of transglutaminases inside the body.

Transglutaminases and the formation of cross-linked proteins in disease

Transglutaminases catalyze nucleophilic substitutions of the carboxamide group of glutaminyl residues [1,2]. The attacking nucleophiles are typically the amines of various compounds, but can include hydroxyl moieties and H_2O depending on the transglutaminase isozyme or conditions. Thus, subject to the nucleophile, transglutaminases catalyze transamidation, esterification, or deamidation of glutaminyl residues. Transamidation involving the ε amine of lysyl residues is the reaction most often catalyzed by transglutaminases and results in the formation of N^{ε}-(γ-glutamyl)lysine isodipeptide linkages between polypeptide chains (Figure 1A). A number of important pathologies exhibit both aberrant transglutaminase activity and increased production of N^{ε}-(γ-glutamyl)lysine cross-linked proteins (*e.g.*, neurodegenerative disorders [3-12] and cardiovascular disease [13-20]). The involvement of increased transglutaminase activity in neurodegenerative or cardiovascular diseases is supported by the observation that genetic inactivation of various transglutaminases in animal models slows progression of these diseases [21-26]. The preceding observation and others prompted a search for medicinal transglutaminase inhibitors [27-29], as well as testing of cystamine in disease models and patients [30-45]. These tests indicate that cystamine might be of benefit in the treatment of selected diseases.

* Present address: Department of Radiology, Weill Cornell Medicine, New York, NY, U.S.A.

Received: 21 May 2018
Revised: 07 November 2018
Accepted: 24 July 2018

Accepted Manuscript Online: 27 July 2018
Version of Record published: 5 September 2018

Bioscience Reports (2018) **38** BSR20180691
https://doi.org/10.1042/BSR20180691

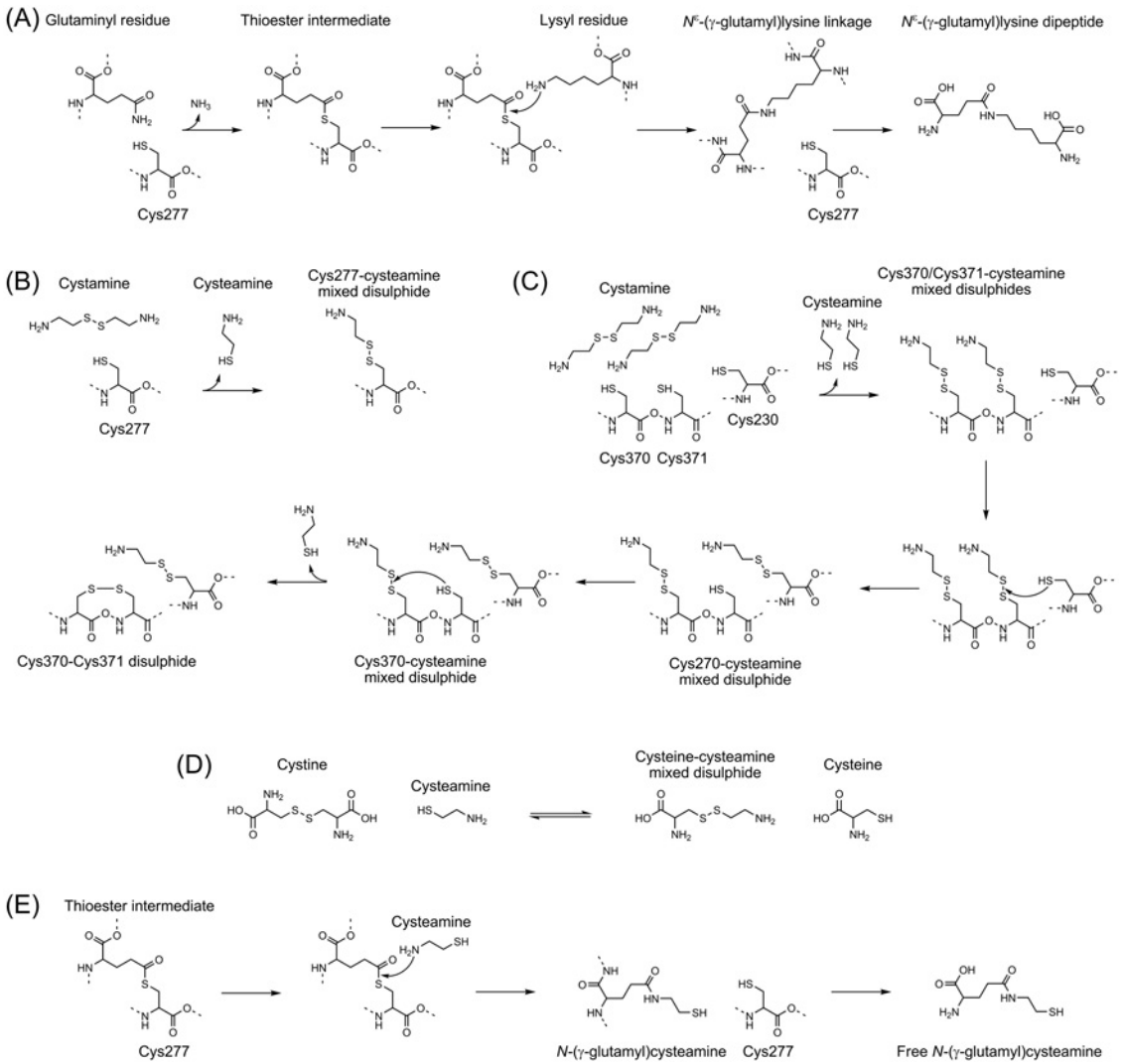

Figure 1. Reactions of cyst(e)amine with transglutaminases and cystine

(**A**) Transglutaminase-catalyzed N^{ε} (γ-glutamyl)lysine isodipeptide formation: transglutaminases catalyze an acyl transfer reaction that proceeds by a Bi-Molecular or Ping-Pong mechanism. Activated transglutaminases first act to form a thioester bond between the active site Cys^{277} and the carboxamide moiety of glutaminyl residues. Formation of this intermediate involves the release of the amide nitrogen as ammonia, which powers the subsequent catalysis. The thioester bond then undergoes a nucleophilic attack by the ε amine of lysine to complete the acyl transfer and produce N^{ε} (γ-glutamyl)lysine isodipeptide linkage. These dipeptides can then be released from the protein by hydrolysis of the peptide linkages. (**B**) Oxidative inactivation of transglutaminase 2 by cystamine by the mechanism of Lorand and Conrad [46]: in this model, the thiol moiety of Cys^{277} participates in thiol-disulphide interchange with cystamine to produce cysteamine–Cys^{277} mixed disulphide. (**C**) Oxidative inactivation of transglutaminase 2 by cysteamine by our interpretation of the mechanism of Palanski and Khosla [48]: in this model, cystamine first forms mixed disulphides with Cys^{370} and Cys^{371}. Cys^{230} then undergoes thiol–disulphide interchange with cysteamine–Cys^{230} mixed disulphide. The newly reduced Cys^{371} then reduces the mixed disulphide of cysteamine–Cys^{370} while being oxidized to the Cys^{370}–Cys^{371} disulphide. It is also possible that the Cys^{230} undergoes thiol–disulphide interchange with the cysteamine–Cys^{370} mixed disulphide rather than the cysteamine–Cys^{371} mixed disulphide. In either case, the Cys^{370}–Cys^{371} disulphide would form and allosterically regulate the enzyme. (**D**) Thiol–disulphide interchange of cysteamine and cystine: cysteamine interacts with cystine by thiol–disulphide interchange to from the cysteamine–cysteine mixed disulphide. Note that the latter resembles the lysyl residue depicted in (A). (**E**) Transglutaminase-catalyzed *N*-(γ-glutamyl)cysteamine formation: a mechanism for the competitive inhibition of transglutaminase by cysteamine. This mechanism is analogous to that shown in (A) and for the sake of brevity begins with thioester bound intermediate. The thio–ester bond is attacked by the amine nitrogen of cysteamine to complete the acyl transfer and produce *N*-(γ-glutamyl)cysteamine. We propose that *N*-(γ-glutamyl)cysteamine is released from the protein by proteolysis, as is the case for other *N*-(γ-glutamyl)amines.

Oxidative mechanisms for the inhibition of transglutaminases by cystamine

Cystamine was first reported to be an inhibitor of transglutaminase 2 by Lorand and Conrad in 1984 [46]. They hypothesized that cystamine and the active site Cys^{277} undergo a thiol-disulphide interchange to produce a mixed disulphide that prevents catalysis (Figure 1B). Cystamine is the disulphide form of cysteamine. Thus, the proposed thiol-disulphide interchange (thiolation) produces free cysteamine and a mixed disulphide of cysteamine and Cys^{277}. An 'oxidative mechanism' for the inhibition of transglutaminase 2 is supported by subsequent investigations by Jeon *et al*. [47], and also by Palanski and Khosla [48]. The latter researchers, however, proposed a modified hypothesis, which states that cystamine forms mixed disulphides with a triad of cysteinyl residues on the surface of transglutaminase 2 that regulates the activation of the extracellular pool of this enzyme. According to Palanski and Khosla [48], cystamine reacts with Cys^{230}, Cys^{370}, or Cys^{371} to promote the eventual formation of an allosteric disulphide bond between Cys^{370} and Cys^{371} as shown in Figure 1C. These mechanisms, however, presume that cystamine is not metabolized *en route* to the targetted transglutaminases; a presumption that is not supported by pharmacokinetic studies.

Conversion of cystamine into cysteamine within the body

Cystamine is rapidly reduced to cysteamine by serum, as well as by the liver and kidneys [49]. By contrast, cysteamine is relatively stable in plasma and rapidly absorbed from blood into tissues [49-53]. Prior to cellular uptake, cysteamine undergoes thiol-disulphide interchange with extracellular cystine to form cysteamine–cysteine mixed disulphide (Figure 1D), which resembles lysine [54,55]. Consequently, the cysteamine–cysteine mixed disulphide enters cells through amino acid transporters and is then reduced to cysteamine and cysteine. Thus, the major form in which cystamine inhibits intracellular transglutaminases is cysteamine and not cystamine.

Cysteamine as an inhibitor of intracellular transglutaminases

In earlier studies, we demonstrated that cysteamine acts as a substrate for transglutaminase 2 to link this compound to glutaminyl residues by way of an isopeptide linkage forming *N*-(γ-glutamyl)cysteamine (Figure 1E) [56]. In other words, cysteamine by virtue of being a transglutaminase 2 substrate, acts as a competitive inhibitor of the other amine substrates of this enzyme. Cystamine has not been shown to be an amine substrate of transglutaminase 2, an assertion erroneously attributed to us elsewhere [48]. Formation of *N*-(γ-glutamyl)cysteamine by transglutaminases could account for two puzzling observations pertaining to the metabolism of exogenously supplied cysteamine. The first of these observations is that a significant portion of the administered cysteamine is unaccounted for following analysis of established routes of metabolism. Cysteamine generated endogenously by the catabolism of pantetheine is oxidized to hypotaurine and then taurine [57]. The administration of cysteamine to rodents, however, does not result in significant accumulation of hypotaurine or taurine in brain or plasma [49], and indicates that the metabolism of exogenous cysteamine bypasses oxidation to taurine. A small portion of cysteamine administered *per os* is metabolized to thialysine and then *S*-(2-aminoethyl)l-cysteine ketimine decarboxylated dimer [50]. Based on the levels of cysteine that accompany cysteamine into cells as a mixed disulphide, significant quantities of cysteamine must enter cells [38,49-51,54,55] but it is then rapidly metabolized. The cellular fate of the majority of exogenous cysteamine remains unaccounted for.

A role for transglutaminases in the metabolism of cysteamine

A novel hypothesis for the metabolism of cysteamine is that it is covalently attached to proteins by intracellular transglutaminases. This hypothesis is supported by the observation that a significant portion of radiolabeled cysteamine administered to animals or cells is covalently bound to proteins, but not by disulphide bonds [58,59]. This hypothesis requires that the intracellular transglutaminases be activated while cysteamine is being absorbed by cells. Transglutaminases are activated by calcium. Exogenous cysteamine may stimulate calcium release from intracellular stores and thereby promote transglutaminase activity. This mechanism depends on the production of hydrogen peroxide (H_2O_2), by micromolar amounts of cysteamine. At these concentrations, thiols (RSH) such as cysteamine reduce transition metals ($M^n \rightarrow M^{n-1}$, where n is the oxidation number), while being oxidized to the corresponding disulphide ($RSSR$):

$$2RSH + 2M^n \rightleftarrows RSSR + 2M^{n-1} + 2H^+$$

Bioscience Reports (2018) **38** BSR20180691
https://doi.org/10.1042/BSR20180691

The reduced metals, in turn, reduce oxygen (O_2) to superoxide (O_2^-):

$$M^{n-1} + O_2 \rightleftarrows M^n + O_2^-$$

Dismutation of superoxide yields hydrogen peroxide (H_2O_2), which is a mild oxidant at physiological pH values (7.2–7.4):

$$2O_2^- + 2H^+ \rightarrow H_2O_2 + O_2$$

Thiols, such as cysteamine, react slowly with hydrogen peroxide under these conditions [60]. Effective scavenging of the peroxide by thiols does not occur until the thiols are present at millimolar concentrations [54]. Thus, at the micromolar concentrations that it attains outside of cells, cysteamine promotes hydrogen peroxide production by the reactions shown above [54]. Hydrogen peroxide readily enters cells and causes a peroxidative stress that is exacerbated by the inhibition of cellular glutathione peroxidases by cysteamine [54].

Hydrogen peroxide promotes the release of calcium from intracellular stores [61,62] and should therefore stimulate transglutaminase activity. In support of this notion, the addition of hydrogen peroxide to cells in culture stimulates their *in situ* transglutaminase activity [63,64].

The above conjecture could be readily tested by investigating the plasma of cysteamine-treated animals or medium of cells in culture treated with cysteamine for the presence of free *N*-(γ-glutamyl)cysteamine. Isopeptide linkages are resistant to proteolysis and consequently transglutaminase-made *N*-(γ-glutamyl)amines are excised as free *N*-(γ-glutamyl)amines during proteolysis of proteins bearing these species [65]. Free *N*-(γ-glutamyl)amines are present in various body fluids and reflect the levels of active transglutaminases in tissues [3,66,67]. If our hypothesis is correct, then the simultaneous measurements of taurine, *S*-(2-aminoethyl)l-cysteine ketimine decarboxylated dimer, as well as protein-bound and free *N*-(γ-glutamyl)cysteamine should provide a comprehensive accounting of the metabolism of exogenous cyst(e)amine, in addition to indicating the mechanism by which cysteamine inhibits intracellular transglutaminases.

Sites for the oxidative inactivation of transglutaminases by cystamine

Transglutaminases are fully activated by the binding of three calcium ions per enzyme and reducing conditions that maintain the active site cysteine in a fully reduced state [1,2,47]. The cytosol is highly reducing and therefore the activation of intracellular transglutaminases is regulated by the availability of cytosolic calcium. The extracellular environment is different; calcium is readily available whereas reductants are not. Khosla et al. discovered that the activity of extracellular transglutaminase 2 is regulated by the redox status of two vicinal cysteinyl residues on the surface of this enzyme [68]. Under the oxidizing conditions of interstitial fluids [69], these residues: Cys^{370} or Cys^{371} form a disulphide in a manner that involves a third cysteinyl residue, Cys^{230}, and ERp57 [70]. Reduction in the Cys^{370}–Cys^{371} disulphide linkage by thioredoxin activates extracellular transglutaminase 2 [71,72]. The activation of extracellular transglutaminase by this mechanism is blocked by cystamine forming mixed disulphides with Cys^{370} and Cys^{371} (Figure 1C). As noted earlier, cystamine is converted into cysteamine in the body [49]. It is possible that a portion of the plasma-derived cysteamine is oxidized to cystamine within the interstitial spaces and in this form inactivates extracellular transglutaminases. The amount of cystamine available to inhibit the extracellular transglutaminases by this mechanism will depend on the amounts of cysteamine and cysteamine–cysteine mixed disulphide; the amounts of the latter are expected to be significant after the administration of cyst(e)amine. It should be noted that cysteamine–cysteine mixed disulphide could also inhibit transglutaminase in an oxidative manner, as shown in Figure 1C with cysteamine–cysteine mixed disulphide replacing cystamine. The lumen of the gut is also likely to be an oxidizing environment because the administration of cysteamine by gavage results in the appearance of cystamine in the plasma [50]; the most likely site for oxidation of cysteamine to cystamine, in this case, is the gut. Thus, cystamine is most likely to inhibit transglutaminases by an oxidative mechanism in the gut. This observation is important since aberrant transglutaminase activities contribute to etiology of several intestinal diseases, in particular, celiac disease. In this disease, transglutaminases act to deamidate glutaminyl residues in the wheat protein gliadin increasing the autoantigenicity of the modified protein in the context of HLA-DQ2 or HLA-DQ8 [73]. Cystamine inhibits the generation of the relevant epitopes *in vitro*, but only at millimolar concentrations [74]. Given the relatively safe use of cysteamine in humans [75-77] and the potential to assess the mechanism by which this compound inhibits transglutaminases (*i.e.*, by measurement of *N*-(γ-glutamyl)cysteamine), cysteamine may be of use in the treatment of celiac disease and other diseases involving transglutaminases.

Conclusion

The activities of intracellular and extracellular transglutaminases contribute to a number of important pathologies. Agents that safely inhibit the *in situ* activities of these transglutaminase pools are therefore of interest as possible therapeutics. The evidence presented here indicates that cystamine inhibits extracellular transglutaminases, while its reduced congener – cysteamine – inhibits intracellular transglutaminases. This distinction is important for the design of other transglutaminase inhibitors based on the mechanisms by which cysteamine or cystamine inhibit these enzymes (*e.g.*, disulphiram [48]). It may also guide the form in which cystamine is administered: as either cystamine or cysteamine. Finally, the measurement of *N*-(γ-glutamyl)cysteamine) may provide a means of determining the mechanism by which intracellular transglutaminases are inhibited following the administration of cystamine or cysteamine.

Competing interests

The authors declare that there are no competing interests associated with the manuscript.

References

1 Jeitner, T.M., Muma, N.A., Battaile, K.P. and Cooper, A.J. (2009) Transglutaminase activation in neurodegenerative diseases. *Future Neurol.* **4**, 449–467, https://doi.org/10.2217/fnl.09.17
2 Klöck, C. and Khosla, C (2012) Regulation of the activities of the mammalian transglutaminase family of enzymes. *Protein Sci.* **21**, 1781–1791, https://doi.org/10.1002/pro.2162
3 Nemes, Z., Fésüs, L., Egerházi, A., Keszthelyi, A. and Degrell, I.M. (2001) N(epsilon)(gamma-glutamyl)lysine in cerebrospinal fluid marks Alzheimer type and vascular dementia. *Neurobiol. Aging* **22**, 403–406, https://doi.org/10.1016/S0197-4580(01)00224-X
4 Zainelli, G.M., Ross, C.A., Troncoso, J.C. and Muma, N.A. (2003) Transglutaminase cross-links in intranuclear inclusions in Huntington disease. *J. Neuropathol. Exp. Neurol.* **62**, 14–24, https://doi.org/10.1093/jnen/62.1.14
5 Andringa, G. et al. (2004) Tissue transglutaminase catalyzes the formation of alpha-synuclein crosslinks in Parkinson's disease. *FASEB J.* **18**, 932–934, https://doi.org/10.1096/fj.03-0829fje
6 Zhang, J. et al. (2016) Tissue transglutaminase and its product isopeptide are increased in Alzheimer's disease and appswe/ps1de9 double transgenic mice brains. *Mol. Neurobiol.* **53**, 5066–5078, https://doi.org/10.1007/s12035-015-9413-x
7 Halverson, R.A., Lewis, J., Frausto, S., Hutton, M. and Muma, N.A. (2005) Tau protein is cross-linked by transglutaminase in P301L tau transgenic mice. *J. Neurosci.* **25**, 1226–1233, https://doi.org/10.1523/JNEUROSCI.3263-04.2005
8 Junn, E., Ronchetti, R.D., Quezado, M.M., Kim, S.Y. and Mouradian, M.M. (2003) Tissue transglutaminase-induced aggregation of alpha-synuclein: Implications for Lewy body formation in Parkinson's disease and dementia with Lewy bodies. *Proc. Natl Acad. Sci. U.S.A.* **100**, 2047–2052, https://doi.org/10.1073/pnas.0438021100
9 Lesort, M., Chun, W., Johnson, G.V. and Ferrante, R.J. (1999) Tissue transglutaminase is increased in Huntington's disease brain. *J. Neurochem.* **73**, 2018–2027
10 Karpuj, M.V. et al. (1999) Transglutaminase aggregates huntingtin into nonamyloidogenic polymers, and its enzymatic activity increases in Huntington's disease brain nuclei. *Proc. Natl Acad. Sci. U.S.A.* **96**, 7388–7393, https://doi.org/10.1073/pnas.96.13.7388
11 Karpuj, M.V., Becher, M.W. and Steinman, L (2002) Evidence for a role for transglutaminase in Huntington's disease and the potential therapeutic implications. *Neurochem. Int.* **40**, 31–36, https://doi.org/10.1016/S0197-0186(01)00060-2
12 Wilhelmus, M.M. et al. (2009) Transglutaminases and transglutaminase-catalyzed cross-links colocalize with the pathological lesions in Alzheimer's disease brain. *Brain Pathol.* **19**, 612–622, https://doi.org/10.1111/j.1750-3639.2008.00197.x
13 Luo, R. et al. (2016) Transglutaminase is a critical link between inflammation and hypertension. *J. Am. Heart Assoc.* **5**, https://doi.org/10.1161/JAHA.116.003730
14 Byrnes, J.R. and Wolberg, A.S. (2016) Newly-recognized roles of factor XIII in thrombosis. *Semin. Thromb. Hemost.* **42**, 445–454, https://doi.org/10.1055/s-0036-1571343
15 de Jager, M. et al. (2015) Tissue transglutaminase-catalysed cross-linking induces Apolipoprotein E multimers inhibiting Apolipoprotein E's protective effects towards amyloid-beta-induced toxicity. *J. Neurochem.* **134**, 1116–1128, https://doi.org/10.1111/jnc.13203
16 de Jager, M. et al. (2016) The blood clotting Factor XIIIa forms unique complexes with amyloid-beta (Aβ) and colocalizes with deposited Aβ in cerebral amyloid angiopathy. *Neuropathol. Appl. Neurobiol.* **42**, 255–272, https://doi.org/10.1111/nan.12244
17 de Jager, M. et al. (2013) Tissue transglutaminase colocalizes with extracellular matrix proteins in cerebral amyloid angiopathy. *Neurobiol. Aging* **34**, 1159–1169, https://doi.org/10.1016/j.neurobiolaging.2012.10.005
18 Chabot, N., Moreau, S., Mulani, A., Moreau, P. and Keillor, J.W. (2010) Fluorescent probes of tissue transglutaminase reveal its association with arterial stiffening. *Chem. Biol.* **17**, 1143–1150, https://doi.org/10.1016/j.chembiol.2010.06.019
19 Matlung, H.L. et al. (2009) Calcification locates to transglutaminases in advanced human atherosclerotic lesions. *Am. J. Pathol.* **175**, 1374–1379, https://doi.org/10.2353/ajpath.2009.090012
20 Cho, B.R. et al. (2008) Increased tissue transglutaminase expression in human atherosclerotic coronary arteries. *Coron. Artery Dis.* **19**, 459–468, https://doi.org/10.1097/MCA.0b013e3283108fc3

21 Mastroberardino, P.G. et al. (2002) 'Tissue' transglutaminase ablation reduces neuronal death and prolongs survival in a mouse model of Huntington's disease. *Cell Death Differ.* **9**, 873–880, https://doi.org/10.1038/sj.cdd.4401093
22 Mattheij, N.J. et al. (2016) Coated platelets function in platelet-dependent fibrin formation via integrin αIIbβ3 and transglutaminase factor XIII. *Haematologica* **101**, 427–436, https://doi.org/10.3324/haematol.2015.131441
23 Beazley, K.E., Reckard, S., Nurminsky, D., Lima, F. and Nurminskaya, M. (2013) Two sides of MGP null arterial disease: chondrogenic lesions dependent on transglutaminase 2 and elastin fragmentation associated with induction of adipsin. *J. Biol. Chem.* **288**, 31400–31408, https://doi.org/10.1074/jbc.M113.495556
24 Matlung, H.L. et al. (2012) Transglutaminase activity regulates atherosclerotic plaque composition at locations exposed to oscillatory shear stress. *Atherosclerosis* **224**, 355–362, https://doi.org/10.1016/j.atherosclerosis.2012.07.044
25 Williams, H. et al. (2010) Effect of transglutaminase 2 (TG2) deficiency on atherosclerotic plaque stability in the apolipoprotein E deficient mouse. *Atherosclerosis* **210**, 94–99, https://doi.org/10.1016/j.atherosclerosis.2009.11.014
26 Pistea, A. et al. (2008) Small artery remodeling and erythrocyte deformability in L-NAME-induced hypertension: role of transglutaminases. *J. Vasc. Res.* **45**, 10–18, https://doi.org/10.1159/000109073
27 Klöck, C., Herrera, Z., Albertelli, M. and Khosla, C. (2014) Discovery of potent and specific dihydroisoxazole inhibitors of human transglutaminase 2. *J. Med. Chem.* **57**, 9042–9064, https://doi.org/10.1021/jm501145a
28 Keillor, J.W., Apperley, K.Y. and Akbar, A (2015) Inhibitors of tissue transglutaminase. *Trends Pharmacol. Sci.* **36**, 32–40, https://doi.org/10.1016/j.tips.2014.10.014
29 McConoughey, S.J. et al. (2010) Inhibition of transglutaminase 2 mitigates transcriptional dysregulation in models of Huntington disease. *EMBO Mol. Med.* **2**, 349–370, https://doi.org/10.1002/emmm.201000084
30 Oh, Y.J. et al. (2017) Role of tissue transglutaminase in age-associated ventricular stiffness. *Amino Acids* **49**, 695–704, https://doi.org/10.1007/s00726-016-2295-z
31 Lin, Y., He, H., Luo, Y., Zhu, T. and Duan, R. (2015) Inhibition of transglutaminase exacerbates polyglutamine-induced neurotoxicity by increasing the aggregation of mutant ataxin-3 in an SCA3 *Drosophila* model. *Neurotox. Res.* **27**, 259–267, https://doi.org/10.1007/s12640-014-9506-8
32 Shin, S. et al. (2013) Transglutaminase type 2 in human abdominal aortic aneurysm is a potential factor in the stabilization of extracellular matrix. *J. Vasc. Surg.* **57**, 1362–1370, https://doi.org/10.1016/j.jvs.2012.09.062
33 Tzang, B.S. et al. (2013) Cystamine ameliorates ventricular hypertrophy associated with modulation of IL-6-mediated signaling in lupus-prone mice. *Life Sci.* **92**, 719–726, https://doi.org/10.1016/j.lfs.2013.01.027
34 Engholm, M., Eftekhari, A., Chwatko, G., Bald, E. and Mulvany, M.J. (2011) Effect of cystamine on blood pressure and vascular characteristics in spontaneously hypertensive rats. *J. Vasc. Res.* **48**, 476–484, https://doi.org/10.1159/000327773
35 Hwang, I.K. et al. (2009) Expression of tissue-type transglutaminase (tTG) and the effect of tTG inhibitor on the hippocampal CA1 region after transient ischemia in gerbils. *Brain Res.* **1263**, 134–142, https://doi.org/10.1016/j.brainres.2009.01.038
36 Eftekhari, A. et al. (2007) Chronic cystamine treatment inhibits small artery remodelling in rats. *J. Vasc. Res.* **44**, 471–482, https://doi.org/10.1159/000106465
37 Wang, X. et al. (2005) Cerebral PET imaging and histological evidence of transglutaminase inhibitor cystamine induced neuroprotection in transgenic R6/2 mouse model of Huntington's disease. *J. Neurol. Sci.* **231**, 57–66, https://doi.org/10.1016/j.jns.2004.12.011
38 Fox, J.H. et al. (2004) Cystamine increases L-cysteine levels in Huntington's disease transgenic mouse brain and in a PC12 model of polyglutamine aggregation. *J. Neurochem.* **91**, 413–422, https://doi.org/10.1111/j.1471-4159.2004.02726.x
39 Dedeoglu, A. et al. (2002) Therapeutic effects of cystamine in a murine model of Huntington's disease. *J. Neurosci.* **22**, 8942–8950, https://doi.org/10.1523/JNEUROSCI.22-20-08942.2002
40 Karpuj, M.V. et al. (2002) Prolonged survival and decreased abnormal movements in transgenic model of Huntington disease, with administration of the transglutaminase inhibitor cystamine. *Nat. Med.* **8**, 143–149, https://doi.org/10.1038/nm0202-143
41 Van Raamsdonk, J.M. et al. (2005) Cystamine treatment is neuroprotective in the YAC128 mouse model of Huntington disease. *J. Neurochem.* **95**, 210–220, https://doi.org/10.1111/j.1471-4159.2005.03357.x
42 Gibrat, C. and Cicchetti, F (2011) Potential of cystamine and cysteamine in the treatment of neurodegenerative diseases. *Prog. Neuropsychopharmacol. Biol. Psychiatry* **35**, 380–389, https://doi.org/10.1016/j.pnpbp.2010.11.023
43 Sun, L. et al. (2010) Effects of cysteamine on MPTP-induced dopaminergic neurodegeneration in mice. *Brain Res.* **1335**, 74–82, https://doi.org/10.1016/j.brainres.2010.03.079
44 Dubinsky, R. and Gray, C. (2006) CYTE-I-HD: phase I dose finding and tolerability study of cysteamine (Cystagon) in Huntington's disease. *Mov. Disord.* **21**, 530–533, https://doi.org/10.1002/mds.20756
45 Prundean, A., Youssov, K., Humbert, S., Bonneau, D. and Verny, C (2015) A phase II, open-label evaluation of cysteamine tolerability in patients with Huntington's disease. *Mov. Disord.* **30**, 288–289, https://doi.org/10.1002/mds.26101
46 Lorand, L. and Conrad, S.M. (1984) Transglutaminases. *Mol. Cell. Biochem.* **58**, 9–35, https://doi.org/10.1007/BF00240602
47 Jeon, J.H. et al. (2004) Different inhibition characteristics of intracellular transglutaminase activity by cystamine and cysteamine. *Exp. Mol. Med.* **36**, 576–581, https://doi.org/10.1038/emm.2004.74
48 Palanski, B.A. and Khosla, C. (2018) Cystamine and disulfiram inhibit human transglutaminase 2 via an oxidative mechanism. *Biochemistry*, https://doi.org/10.1021/acs.biochem.8b00204
49 Pinto, J.T. et al. (2005) Treatment of YAC128 mice and their wild-type littermates with cystamine does not lead to its accumulation in plasma or brain: implications for the treatment of Huntington disease. *J. Neurochem.* **94**, 1087–1101, https://doi.org/10.1111/j.1471-4159.2005.03255.x

50 Pinto, J.T. et al. (2009) Measurement of sulfur-containing compounds involved in the metabolism and transport of cysteamine and cystamine. Regional differences in cerebral metabolism. *J. Chromatogr. B Analyt. Technol. Biomed. Life Sci.* **877**, 3434–3441, https://doi.org/10.1016/j.jchromb.2009.05.041
51 Bousquet, M. et al. (2010) Cystamine metabolism and brain transport properties: clinical implications for neurodegenerative diseases. *J. Neurochem.* **114**, 1651–1658, https://doi.org/10.1111/j.1471-4159.2010.06874.x
52 Dohil, R., Cabrera, B.L., Gangoiti, J.A., Barshop, B.A. and Rioux, P. (2014) Pharmacokinetics of cysteamine bitartrate following intraduodenal delivery. *Fundam. Clin. Pharmacol.* **28**, 136–143, https://doi.org/10.1111/fcp.12009
53 Bouazza, N. et al. (2011) Population pharmacokinetics and pharmacodynamics of cysteamine in nephropathic cystinosis patients. *Orphanet J. Rare Dis.* **6**, 86, https://doi.org/10.1186/1750-1172-6-86
54 Jeitner, T.M. and Lawrence, D.A. (2001) Mechanisms for the cytotoxicity of cysteamine. *Toxicol. Sci.* **63**, 57–64, https://doi.org/10.1093/toxsci/63.1.57
55 Meier, T. and Issels, R.D. (1995) Promotion of cyst(e)ine uptake. *Methods Enzymol.* **252**, 103–112, https://doi.org/10.1016/0076-6879(95)52013-9
56 Jeitner, T.M., Delikatny, E.J., Ahlqvist, J., Capper, H. and Cooper, A.J. (2005) Mechanism for the inhibition of transglutaminase 2 by cystamine. *Biochem. Pharmacol.* **69**, 961–970, https://doi.org/10.1016/j.bcp.2004.12.011
57 Jacobsen, J.G. and Smith, L.H. (1968) Biochemistry and physiology of taurine and taurine derivatives. *Physiol. Rev.* **48**, 424–511, https://doi.org/10.1152/physrev.1968.48.2.424
58 Modig, H.G., Edgren, M. and Révész, L (1972) Release of thiols from cellular mixed disulphides and its possible role in radiation protection. *Int. J. Radiat. Biol. Relat. Stud. Phys. Chem. Med.* **22**, 257–268, https://doi.org/10.1080/09553007214551031
59 Power, J.A., Goldstein, L.S. and Harris, J.W. (1974) Letter: a test of the 'mixed-disulphide' hypothesis of cysteamine radioprotection. *Int. J. Radiat. Biol. Relat. Stud. Phys. Chem. Med.* **26**, 91–96, https://doi.org/10.1080/09553007414551011
60 Winterbourn, C.C. and Metodiewa, D (1999) Reactivity of biologically important thiol compounds with superoxide and hydrogen peroxide. *Free Radic. Biol. Med.* **27**, 322–328, https://doi.org/10.1016/S0891-5849(99)00051-9
61 Gibson, G.E., Zhang, H., Xu, H., Park, L.C. and Jeitner, T.M. (2002) Oxidative stress increases internal calcium stores and reduces a key mitochondrial enzyme. *Biochim. Biophys. Acta* **1586**, 177–189, https://doi.org/10.1016/S0925-4439(01)00091-6
62 Wang, X., Takeda, S., Mochizuki, S., Jindal, R. and Dhalla, N.S. (1999) Mechanisms of hydrogen peroxide-induced increase in intracellular calcium in cardiomyocytes. *J. Cardiovasc. Pharmacol. Ther.* **4**, 41–48, https://doi.org/10.1177/107424849900400107
63 Lee, Z.W. et al. (2003) Activation of *in situ* tissue transglutaminase by intracellular reactive oxygen species. *Biochem. Biophys. Res. Commun.* **305**, 633–640, https://doi.org/10.1016/S0006-291X(03)00835-0
64 Yi, S.J. et al. (2004) Arachidonic acid activates tissue transglutaminase and stress fiber formation via intracellular reactive oxygen species. *Biochem. Biophys. Res. Commun.* **325**, 819–826, https://doi.org/10.1016/j.bbrc.2004.10.122
65 Jeitner, T.M., Battaile, K. and Cooper, A.J. (2013) γ-Glutamylamines and neurodegenerative diseases. *Amino Acids* **44**, 129–142, https://doi.org/10.1007/s00726-011-1209-3
66 Jeitner, T.M. et al. (2001) N(epsilon)-(gamma-L-glutamyl)-L-lysine (GGEL) is increased in cerebrospinal fluid of patients with Huntington's disease. *J. Neurochem.* **79**, 1109–1112, https://doi.org/10.1046/j.1471-4159.2001.00673.x
67 Jeitner, T.M., Matson, W.R., Folk, J.E., Blass, J.P. and Cooper, A.J. (2008) Increased levels of gamma-glutamylamines in Huntington disease CSF. *J. Neurochem.* **106**, 37–44, https://doi.org/10.1111/j.1471-4159.2008.05350.x
68 Stamnaes, J., Pinkas, D.M., Fleckenstein, B., Khosla, C. and Sollid, L.M. (2010) Redox regulation of transglutaminase 2 activity. *J. Biol. Chem.* **285**, 25402–25409, https://doi.org/10.1074/jbc.M109.097162
69 Yi, M.C. and Khosla, C. (2016) Thiol-disulfide exchange reactions in the mammalian extracellular environment. *Annu. Rev. Chem. Biomol. Eng.* **7**, 197–222, https://doi.org/10.1146/annurev-chembioeng-080615-033553
70 Yi, M.C., Melkonian, A.V., Ousey, J.A. and Khosla, C. (2018) Endoplasmic reticulum-resident protein 57 (ERp57) oxidatively inactivates human transglutaminase 2. *J. Biol. Chem.* **293**, 2640–2649, https://doi.org/10.1074/jbc.RA117.001382
71 Plugis, N.M., Palanski, B.A., Weng, C.H., Albertelli, M. and Khosla, C. (2017) Thioredoxin-1 selectively activates transglutaminase 2 in the extracellular matrix of the small intestine: implications for celiac disease. *J. Biol. Chem.* **292**, 2000–2008, https://doi.org/10.1074/jbc.M116.767988
72 Jin, X. et al. (2011) Activation of extracellular transglutaminase 2 by thioredoxin. *J. Biol. Chem.* **286**, 37866–37873, https://doi.org/10.1074/jbc.M111.287490
73 Hadjivassiliou, M. et al. (2010) Gluten sensitivity: from gut to brain. *Lancet Neurol.* **9**, 318–330, https://doi.org/10.1016/S1474-4422(09)70290-X
74 Molberg, O. et al. (2001) T cells from celiac disease lesions recognize gliadin epitopes deamidated in situ by endogenous tissue transglutaminase. *Eur. J. Immunol.* **31**, 1317–1323, https://doi.org/10.1002/1521-4141(200105)31:5%3c1317::AID-IMMU1317%3e3.0.CO;2-I
75 Langman, C.B. et al. (2012) A randomized controlled crossover trial with delayed-release cysteamine bitartrate in nephropathic cystinosis: effectiveness on white blood cell cystine levels and comparison of safety. *Clin. J. Am. Soc. Nephrol.* **7**, 1112–1120, https://doi.org/10.2215/CJN.12321211
76 Dohil, R. and Cabrera, B.L. (2013) Treatment of cystinosis with delayed-release cysteamine: 6-year follow-up. *Pediatr. Nephrol.* **28**, 507–510, https://doi.org/10.1007/s00467-012-2315-5
77 Medic, G., van der Weijden, M., Karabis, A. and Hemels, M. (2017) A systematic literature review of cysteamine bitartrate in the treatment of nephropathic cystinosis. *Curr. Med. Res. Opin.* **33**, 2065–2076, https://doi.org/10.1080/03007995.2017.1354288