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Mechanism of action of the disease-modifying anti-arthritic thiol agents D-penicillamine and sodium aurothiomalate: Restoration of cellular free thiols and sequestration of reactive aldehydes

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Abstract

While new anti-cytokine disease-modifying anti-arthritic drugs for rheumatoid arthritis have been designed via mechanistic approaches, the mechanism of action of a number of more established disease-modifying anti-arthritic drugs has not been elucidated. In the case of D-penicillamine and sodium aurothiomalate, the key structural feature appears to be a free thiol group. However, the role thiol groups play in the therapeutic efficacy of these drugs has not been defined. A number of lines of evidence have demonstrated increased generation of reactive aldehydes and the associated depletion of free thiol pools in rheumatoid arthritis. These observations have led to the suggestion that reactive aldehydes may be the ultimate mediators of cell destruction in rheumatoid arthritis joints. Our data clearly demonstrate that thiol-containing disease-modifying anti-arthritic agents both directly sequester reactive aldehydes and augment intracellular thiol pools, which also can buffer increased aldehyde load and oxidative stress. These data are consistent with clinical data that penicillamine lowers synovial aldehyde levels and augments plasma thiols. We suggest that these actions are the pivotal mechanism of action of thiol-containing disease-modifying anti-arthritic drugs. Understanding the mechanism of action of these drugs provides the opportunity for the design of more potent and safer thiol drug candidates.

Keywords: Rheumatoid arthritis; D-penicillamine; Thiol; Aldehyde sequestration; Cytoprotection

1. Introduction

Despite many decades of research, the mechanism of action of the disease-modifying anti-rheumatic drugs penicillamine and sodium aurothiomalate has remained elusive. Several cogent reviews (Drury et al., 1984; Jellum et al., 1977) have concluded that the key structural feature of these drugs is a free thiol group. With respect to gold-containing drugs, only gold complexed to thiol compounds is effective; gold chloride and colloidal gold are ineffective for the treatment of rheumatoid arthritis (Drury et al., 1984). In the case of sodium aurothiomalate, this disease-modifying anti-arthritic drugs dissociates in plasma to free thiomalate and protein bound gold (Rudge et al., 1984; Jellum et al., 1979). Other thiol agents have also been reported to demonstrate anti-rheumatic activity and include captopril (Drury et al., 1984; Martin et al., 1984) but not the non-thiol ACE inhibitor pentopril (Bird et al., 1990); bucillamine (Yokota et al., 2007); tiopronin (Drury et al., 1984); and 2-oxo-3-(2-mercaptoethyl)-5-phenylimadazolidine, the major thiol metabolite of levamisole (Drury et al., 1984; Kumar et al., 1980; De Brabander et al., 1979; Table 1). Despite evidence for the requirement of a thiol group in these drugs, the functional role of the thiol group in therapeutic efficacy has not been defined. It clearly does not involve antioxidant activity since these disease-modifying anti-arthritic agents are not better antioxidants than trolox or ascorbic acid (Mazor et al., 2006), potent antioxidants that do not demonstrate anti-rheumatic activity.

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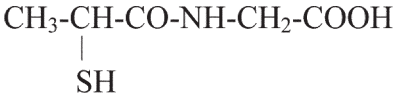
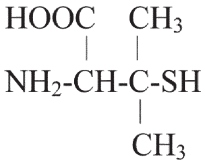
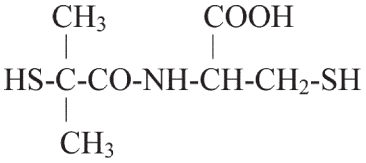
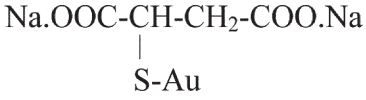
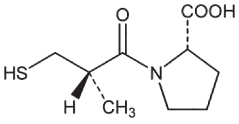
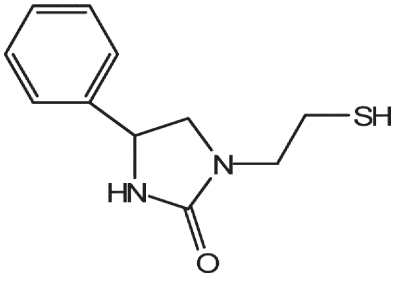
Table 1
Structures of thiol-containing DMARD drugs

Thiol drug	Structure
Tiopronin (Drury et al., 1984; Wood et al., 2007b)	
Penicillamine (Drury et al., 1984; Jellum et al., 1977)	
Bucillamine (Yokota et al., 2007; Wielandt et al., 2006)	
Sodium aurothiomalate (Drury et al., 1984; Jellum et al., 1977)	
Thiomalic acid (Drury et al., 1984; Jellum et al., 1977)	
Captopril (Martin et al., 1984)	
Levamisole metabolite (Kumar et al., 1980; DeBrabander et al., 1979)	

Hallmark features of thiol agents that might explain their therapeutic actions as disease-modifying anti-arthritic drugs are their avidity to sequester cytotoxic aldehydes (Ivanova et al., 2002; Wood et al., 2006a; Nagasawa et al., 1978; Wondrak et al., 2002; Zeng and Davies, 2006; Esterbauer et al., 1976) and to restore cellular thiol pools via stimulating cystine uptake (Issels et al., 1988; Jokay et al., 1998), disulfide exchange reactions (Gahl et al., 1985; Wood et al., 2007b), and stimulation of glutathione synthesis (Issels et al., 1988; Jokay et al., 1998; Wielandt et al., 2006). These pharmacological actions are extremely relevant since free thiols are decreased in the plasma (Giustarini et al., 2005; Banford et al., 1982) and cytotoxic aldehyde products of lipid peroxidation are increased in plasma and synovial fluid (Wade et al., 1987) of RA patients. Increased polyamine oxidase activity in synovial fluid and synovial tissues (Ferrante et al., 1990; Seiler, 2004) results in augmented production of putrescine in rheumatoid arthritis (Yukioka et al., 1992). Coupled with the production of putrescine is the generation of the cytotoxic aldehydes acrolein and 3-aminopropanal (Ivanova et al., 2002; Seiler, 2004). Cytotoxic aldehydes produced by synoviocytes *in vitro* can mediate collagen degradation (Morquette et al., 2006; Tiku et al., 2003).

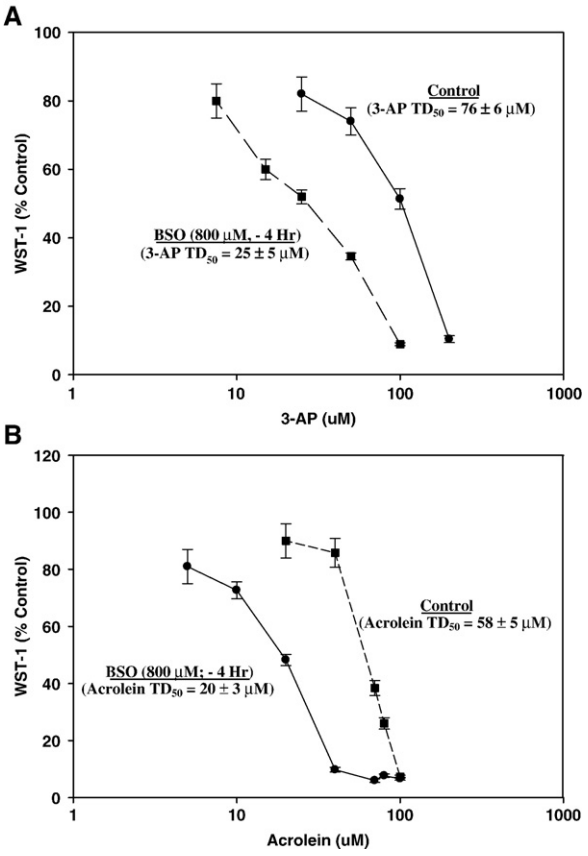


Fig. 1. Concentration-dependent cell death of SN56 cells by the reactive aldehydes 3-aminopropanal (3-AP) (A) and acrolein (B). Prior reduction of cellular glutathione with buthionine sulfoximine (BSO, 800 μM, 4 h prior to aldehyde addition) potentiated aldehyde-dependent cell death. Data are presented as % control ± S.E.M. (n = 6 wells) based on the WST-1 assay at 24 h post-aldehyde addition. This experiment was repeated 3 times.

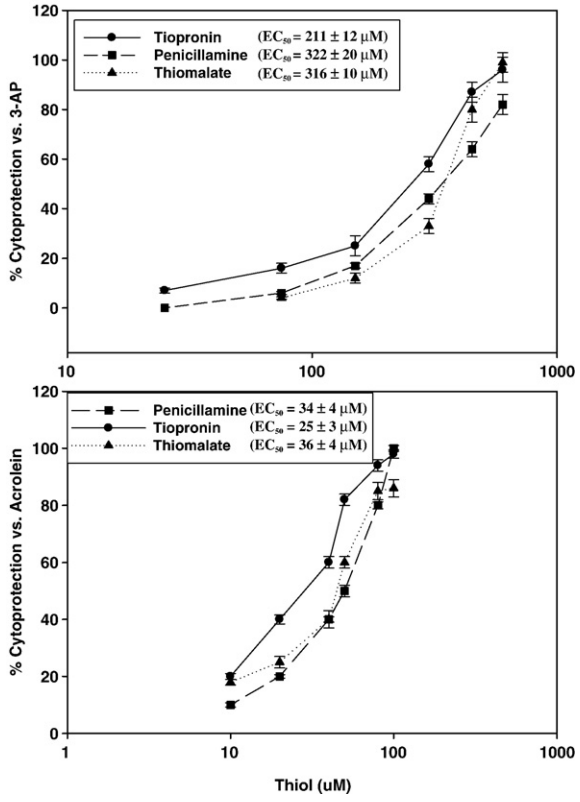


Fig. 2. Concentration-dependent antagonism, with D-penicillamine, tiopronin and thiomalate, of SN56 cell death by the reactive aldehydes 3-aminopropanal (3-AP, 400 μM) and acrolein (100 μM). Data are presented as % cytoprotection ± S.E.M. (n = 6 wells) based on the WST-1 assay at 24 h post-aldehyde addition. The drugs were added as co-treatments. This experiment was repeated 3 times.

In light of these congruent findings, we decided to investigate the ability of thiol-containing disease-modifying anti-arthritic drugs to protect cells from acrolein and 3-aminopropanal cytotoxicity, to characterize the thiol–aldehyde adducts and to examine the ability of thiol-containing disease-modifying anti-arthritic drugs to augment cellular free thiols.

2. Materials and methods

2.1. Cell culture

The murine cell line, SN56.B5.G4 (Blusztajn et al., 1992) was grown in 75 cm^2 flasks in DMEM/Ham's F12 (1:1) containing 10% FBS. For aldehyde toxicity experiments cells were harvested and grown in 48 well plates. For biochemical studies cells were grown in 12 well plates.

2.2. Aldehyde toxicity

Aldehyde-induced cell death was examined with the α,β-unsaturated aldehyde, acrolein and the amino aldehyde, 3-aminopropanal. Aldehyde concentration response data were generated for inducing cell death in control cells and cells treated with buthionine sulfoximine (800 μM) which lowers intracellular glutathione levels by 50 to 60% (McNutt-Scott and Harris, 1998). For drug protection studies, aldehyde concentrations that elicited 100% cell death were used: acrolein (100 μM) and 3-aminopropanal (400 μM). Thiomalate, D-penicillamine and tiopronin were added as co-incubations. For

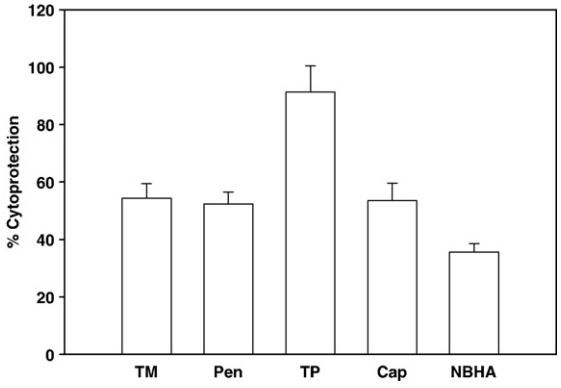


Fig. 3. SN56 cell death induced by 3-aminopropanal (400 μM) is antagonized by delayed (2.5 h) addition of thiomalate (TM), D-penicillamine (Pen), tiopronin (TP), captopril (Cap) and N-benzylhydroxylamine (NBHA), all at 500 μM. Data are presented as % cytoprotection ± S.E.M. (n = 6 wells) based on the WST-1 assay at 24 h post-aldehyde addition. This experiment was repeated 2 times. All drug additions are significantly different (p < 0.05) from 3-aminopropanal + vehicle (i.e. 100% cell death or 0% cytoprotection).

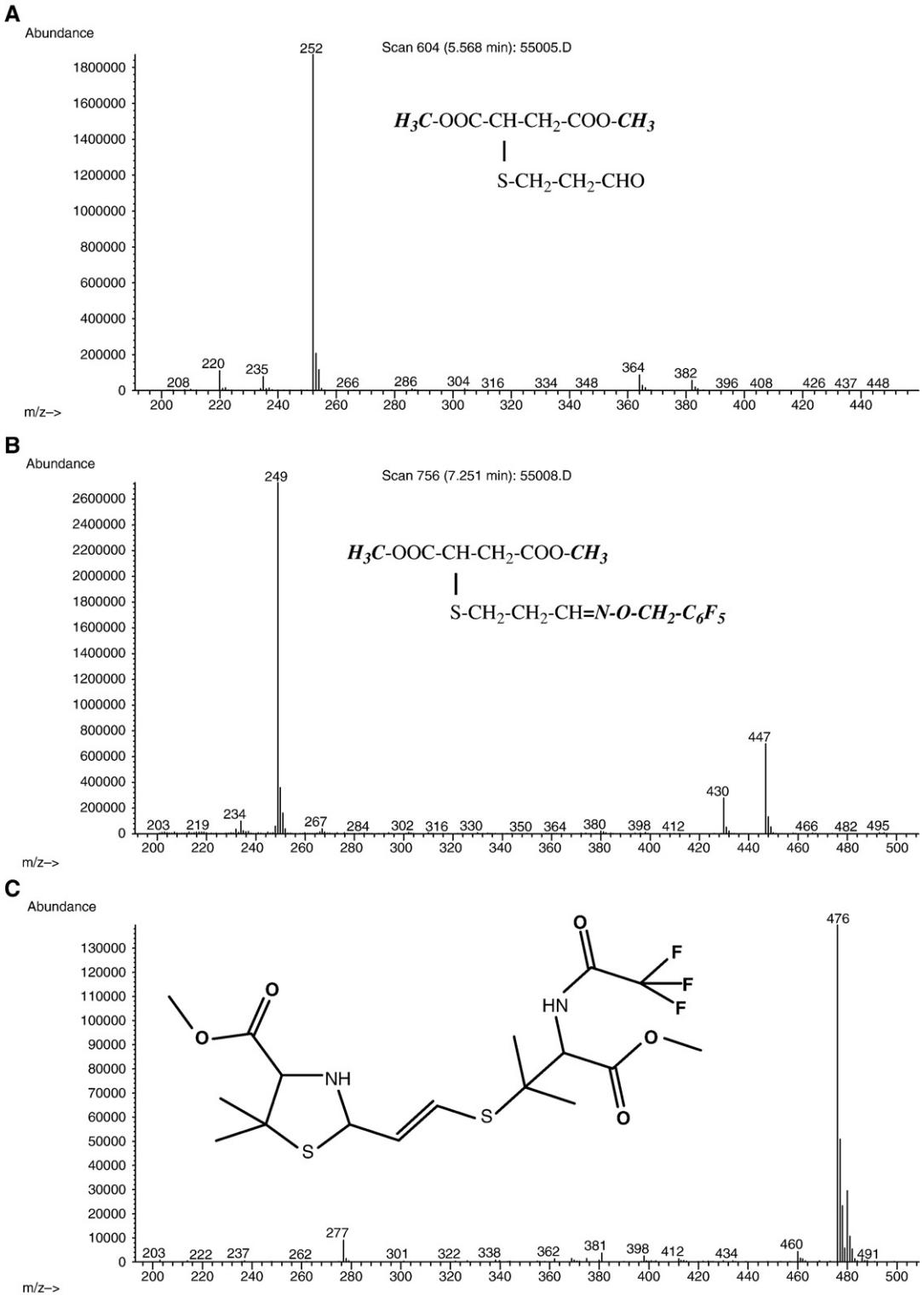


Fig. 4. Mass spectra of the thiol–aldehyde adduct (M.Wt.=206) of thiomalate and acrolein. The 1:1 adduct was first methylated (2 M HCl/methanol at 80 °C for 1 h) and the reaction product (M.Wt.=234) yielded a strong $[M+18]^+$ ion of 252 and a small $[MH]^+$ ion of 235 in ammonia PCI (A). This product was further derivatized with pentaflurobenzylhydroxylamine to yield the oxime derivative (M.Wt.=429, B) with the following key ions: $[MH]^+$ of 430, $[M+18]^+$ of 447, and $[MH-181]^+$ of 249. Penicillamine formed the cyclized adduct of 1 mole acrolein and 2 moles penicillamine (M.Wt.=348). This product was methylated and acylated with trifluoroacetic anhydride. The derivative (M.Wt.=458) formed a strong $[M+18]^+$ ion of 476 (C).

delayed drug addition studies, thiomalate, D-penicillamine, tiopronin, captopril, and *N*-benzylhydroxylamine were administered 2.5 h after 3-aminopropanal. After 24 h, cell viability was assayed with the Roche WST-1 assay kit.

2.3. Cellular cysteine, cystine and cystathionine

Cells were treated with 50 to 600 μM tiopronin, thiomalate or D-penicillamine for 24 h and cellular thiols measured.

Cellular cysteine was quantitated by GC-MS, using deuterated internal standards, as previously described (Wood et al., 2006b, 2007b). Cystine and cystathionine were measured by GC-MS after methylation and acylation with pentafluorpropionic anhydride (Wood and Cheney, 1985). In ammonia PCI the $[M+ 18]^+$ ions that were monitored were 560 (cystathionine), 564 ($[^2H_4]$cystathionine), 578 (cystine) and 582 ($[^2H_4]$cystine). Levels were expressed as nmol/mg of acid-precipitable protein (1 N HCl; 25,000 ×g).

2.4. Thiomaleate- and penicillamine-acrolein adduct formation

To monitor for the predicted (Esterbauer et al., 1976) thiazolidine diadduct product (M.Wt.=334) of 2 moles of penicillamine and 1 mole of acrolein, product formation in PBS was first monitored. The reaction mix was Savant dried after 2 h and methylated. The methylated product was Savant dried and the dimethyl-monoacyl derivative (M.Wt.=458) formed with trifluoroacetic anhydride monitored by GC-MS. This derivative formed a strong $[MH+ \text{ ammonia}]^+$ ion of 476.

To determine the mass spectra of the reaction of thiomalate and acrolein, the 2 h reaction mix was Savant dried and then methylated. A single dimethylated reaction product (M.Wt.=234) was observed which was the monoadduct with a strong $[MH+ \text{ ammonia}]^+$ ion of 252 and a small $[MH]^+$ ion of 235 in ammonia PCI. This product was further derivatized with pentaflurobenzylhydroxylamine to yield the oxime with the following key ions: $[MH]^+$ of 430, $[MH+ \text{ ammonia}]^+$ of 447, and $[M- 181]^+$ of 249. To further validate this adduct, we loaded cells (75 cm^2 flask) with 300 μM thiomalate for 1 h and then added 100 μM acrolein and incubated the cells for a further 2 h. Cellular extracts were methylated and yielded the anticipated $[MH+ \text{ ammonia}]^+$ ion of 252.

2.5. Statistics

All experimental groups consisted of 6 tissue culture wells and all experiments were repeated at least 2 times. Data were analyzed with a one-way ANOVA followed by the Dunnett's *t* test.

3. Results

3.1. Aldehyde-dependent cell death in SN56 cells

We have previously demonstrated that acrolein and 3-aminopropanal, cytotoxic aldehyde products of augmented polyamine metabolism, reduce intracellular free thiols in SN56 cells and that thiol agents can restore intracellular thiol status and protect cells from aldehyde-mediated cell death (Wood et al., 2007b). We utlized this well characterized cell line to further investigate the actions of thiol-containing disease-modifying anti-arthritic drugs. Acrolein and 3-aminopropanal demonstrated concentration-dependent cell death with a 24 h incubation period (Fig. 1). Aldehyde mediated cell death was significantly potentiated (3-fold increase in aldehyde potency) by prior reduction of glutathione levels with buthionine sulfoximine (BSO), a selective inhibitor of γ-glutamylcysteine ligase (EC 6.3.2.2; McNutt-Scott and Harris, 1998), the rate-limiting enzyme, in glutathione biosynthesis (Fig. 1).

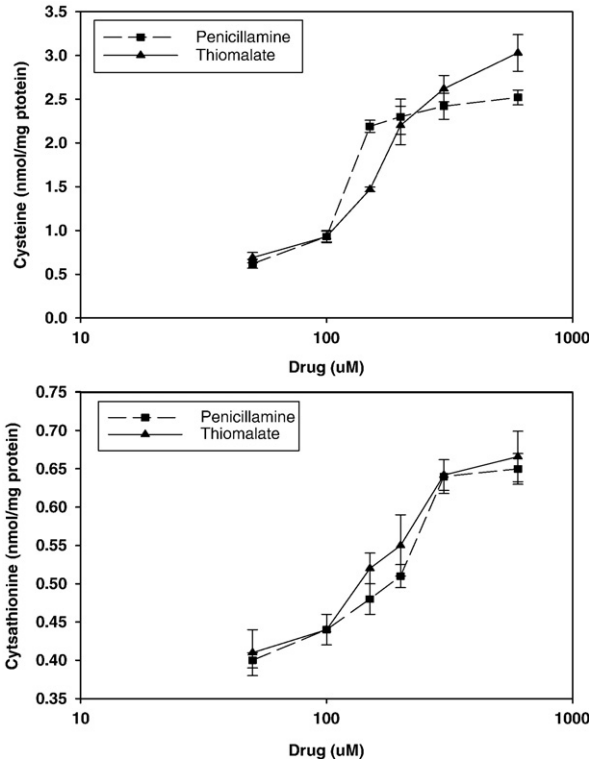


Fig. 5. Augmentation of intracellular cysteine and cystathionine levels in SN56 cells after 6 h treatments with D-penicillamine or thiomalate. Data are presented as mean±S.E.M. (n=6 wells). All drug effects were significant ($p<0.05$) at ≥100 μM. This experiment was repeated 2 times.

3.2. Cytoprotection against aldehyde-dependent cell death with disease-modifying anti-arthritic thiol agents

Using aldehyde concentrations that resulted in 100% cell death we were able to show concentration-dependent cytoprotection (Fig. 2) with D-penicillamine, tiopronin, and thiomalate. Next we investigated the ability of delayed addition of thiols to protect SN56 cells from 3-aminopropanal cytotoxicity (Fig. 3). All the thiol agents examined provided at least 55% cytoprotection with a delayed addition 2.5 h after the aldehyde. The reference aldehyde sequestering agent, *N*-benzylhydroxylamine (Wood et al., 2007a) also provided cytoprotection with delayed administration. The potent antioxidant, trolox (200 and 600 μM) was ineffective as a cytoprotectant in this assay (data not shown).

3.3. Aldehyde–thiol adducts

To demonstrate direct aldehyde sequestration by thioamalate and D-penicillamine, we analyzed, by GC-MS, the adducts formed with acrolein (Fig. 4). Penicillamine and thiomalate can theoretically form hemithioacetals with reactive aldehydes. Penicillamine (Nagasawa et al., 1978; Wondrak et al., 2002), like cysteine (Esterbauer et al., 1976) should also further cyclize to form a thiazolidine ring. This indeed was the case. The product (M.Wt=334) of D-penicillamine and acrolein was derivatized to

obtain the volatile dimethyl-monoacyl derivative (M.Wt.=458) that yielded an intense [MH+ ammonia]$^+$ ion of 476. The reaction product (M.Wt.=206) of thiomalate and acrolein was derivatized to obtain the volatile dimethyl product (M.Wt.=234) which on GC-MS yielded a strong [MH+ ammonia]$^+$ ion of 252. The dimethyl product was further derivatized with pentaflurobenzyl-hydroxylamine to yield the oxime derivative (M.Wt.=429) with the following key ions: [MH]$^+$ of 430, [MH+ ammonia]$^+$ of 447, and [MH− 181]$^+$ of 249. These data support the ability of D-penicillamine and thiomalate to directly sequester cytotoxic aldehydes.

3.4. Disease-modifying anti-arthritic thiol agents augment intracellular cysteine levels

Next we investigated the ability of disease-modifying anti-arthritic thiol agents to augment intracellular cysteine levels (Fig. 5). We have previously demonstrated that tiopronin can augment intracellular cysteine levels via disulfide exchange reactions (Wood et al., 2007b). Similarly we found that penicillamine and thiomalate increased intracellular cysteine levels in a concentration-dependent manner (Fig. 5). This increased availability of cysteine also resulted in augmented synthesis of cystathionine (Fig. 5). Intracellular cystine levels were increased 15 to 20% at all penicillamine and thiomalate concentrations, consistent with rapid metabolism of cystine to cysteine (Wood et al., 2007b) and maintenance of low intracellular cystine levels.

4. Discussion

Our data demonstrate clearly that disease-modifying anti-arthritic thiol agents act to both directly sequester reactive aldehydes and to restore intracellular thiol pools that can also act to sequester aldehydes. These data are consistent with the observations that penicillamine can form adducts with reactive glycoaldehydes (Wondrak et al., 2002; Zeng and Davies, 2006) and acetaldehyde (Nagasawa et al., 1978), that penicillamine decreases aldehyde load in synovial fluid of rheumatoid arthritis patients (Wade et al., 1987), and that penicillamine and sodium aurothiomalate augment plasma free thiol levels that are decreased in rheumatoid arthritis patients (Banford et al., 1982; Haataja et al., 1978). Increased synovial aldehydes in rheumatoid arthritis include malondialdehyde (Wade et al., 1987), glycoaldehydes (Chen et al., 1998), and presumably acrolein and 3-aminopropanal, byproducts of increased polyamine metabolism (Morquette et al., 2006; Tiku et al., 2003; Ferrante et al., 1990). Reactive aldehydes produced by chondrocytes, synoviocytes and invading immune cells bind to collagen making it more prone to degradation (Morquette et al., 2006; Tiku et al., 2003), leading to destruction of articular cartilage and ultimately to deterioration of subchondral bone. The aminoaldehydes produced from polyamine metabolism also are lysomotropic (Wood et al., 2007a; Yu et al., 2003) and release proteolytic cathepsins, like cathepsin D which is known to be increased in the extracellular space in rheumatoid arthritis (Dingle, 1975) and participate in local tissue destruction. While immune-mediated inflammatory cascades are early events in rheumatoid arthritis, inflammatory mediators augment polyamine metabolism and aldehyde production which in turn feedback and further augment ongoing inflammation (Furumitsu et al., 2000), a vicious cycle that can be broken by thiol agents (Kumar et al., 1980; De Brabander et al., 1979; Wielandt et al., 2006; Peristeris et al., 1992). These data are all consistent with the concept that reactive aldehydes may be the critical mediators of cell death in rheumatoid arthritis.

In toto, current scientific data suggest that reactive aldehydes may be the final common mediators of cell death in rheumatoid arthritis joint destruction. Our data further suggest that disease-modifying anti-arthritic thiol agents act to reduce this aldehyde load and slow joint injury. The clinical efficacy of thiol-containing disease-modifying anti-arthritic agents may well require the additive actions of aldehyde sequestration, free radical scavenging (Kumar et al., 1980; Mazor et al., 2006), and down-regulation of pro-inflammatory cascades (Peristeris et al., 1992; Wielandt et al., 2006; Yokota et al., 2007). Understanding the mechanism of action of disease-modifying anti-arthritic thiols allows for the design of more effective thiol agents with greater therapeutic indices.

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References

Banford, J.C., Brown, D.H., Hazelton, R.A., McNeil, C.J., Smith, W.E., Sturrock, R.D., 1982. Altered thiol status in patients with rheumatoid arthritis. Rheumatol. Int. 2, 107–111.

Bird, H.A., Le Gallez, P., Dixon, J.S., Catalano, M.A., Traficante, A., Liauw, L.A., Sussman, H., Rotman, H., Wright, V., 1990. A clinical and biochemical assessment of a nonthiol ACE inhibitor (pentopril; CGS-13945) in active rheumatoid arthritis. J. Rheumatol. 17, 603–608.

Blusztajn, J.K., Venturini, A., Jackson, D.A., Lee, H.J., Wainer, B.H., 1992. Acetylcholine synthesis and release is enhanced by dibutyryl cyclic AMP in a neuronal cell line derived from mouse septum. J. Neurosci. 12, 793–799.

Chen, J.R., Takahashi, M., Suzuki, M., Kushida, K., Miyamoto, S., Inoue, T., 1998. Pentosidine in synovial fluid in osteoarthritis and rheumatoid arthritis: relationship with disease activity in rheumatoid arthritis. J. Rheumatol. 25, 2440–2444.

De Brabander, M., Van Belle, H., Aerts, F., Van De Veire, R., Geuens, G., 1979. Protective effect of levamisole and its sulfhydryl metabolite OMPI against cell death induced by glutathione depletion. Int. J. Immunopharmacol. 1, 93–100.

Dingle, J.T., 1975. The secretion of enzymes into the pericellular environment. Philos. Trans. R Soc. Lond. B Biol. Sci. 271, 315–324.

Drury, P.L., Rudge, S.R., Perrett, D., 1984. Structural requirements for activity of certain 'specific' antirheumatic drugs: more than a simple thiol group? Br. J. Rheumatol. 23, 100–106.

Esterbauer, H., Ertl, A., Scholz, N., 1976. The reaction of cysteine with α,β-unsaturated aldehydes. Tetrahedron 32, 285–289.

Ferrante, A., Storer, R.J., Cleland, L.J., 1990. Polyamine oxidase activity in rheumatoid arthritis synovial fluid. Clin. Exp. Immunol. 80, 373–375.

Furumitsu, Y., Yukioka, K., Yukioka, M., Ochi, T., Morishima, Y., Matsui-Yuasa, I., Otani, S., Inaba, M., Nishizawa, Y., Morii, H., 2000. Interleukin-1beta induces elevation of spermidine/spermine N1-acetyltransferase activity and an increase in the amount of putrescine in synovial adherent cells from patients with rheumatoid arthritis. J. Rheumatol. 27, 1352–1357.

Gahl, W.A., Tietze, F., Butler, J.D., Schulman, J.D., 1985. Cysteamine depletes cystinotic leucocyte granular fractions of cystine by the mechanism of disulphide interchange. Biochem. J. 228, 545–550.

Giustarini, D., Lorenzini, S., Rossi, R., Chindamo, D., Di Simplicio, P., Marcolongo, R., 2005. Altered thiol pattern in plasma of subjects affected by rheumatoid arthritis. Clin. Exp. Rheumatol. 23, 205–212.

Haataja, M., Nissila, M., Ruutsalo, H.M., 1978. Serum sulfhydryl levels in rheumatoid patients treated with gold thiomalate and penicillamine. Scand. J. Rheumatol. 7, 212–214.

Issels, R.D., Nagele, A., Eckert, K.G., Wilmanns, W., 1988. Promotion of cystine uptake and its utilization for glutathione biosynthesis induced by cysteamine and *N*-acetylcysteine. Biochem. Pharmacol. 37, 881–888.

Ivanova, S., Batliwalla, F., Mocco, J., Kiss, S., Huang, J., Mack, W., Coon, A., Eaton, J.W., Al-Abed, Y., Gregersen, P.K., Shohami, E., Connolly Jr., E.S., Tracey, K.J., 2002. Neuroprotection in cerebral ischemia by neutralization of 3-aminopropanal. Proc. Natl. Acad. Sci. U S A. 99, 5579–5584.

Jellum, E., Aaseth, J., Munthe, E., 1977. Is the mechanism of action during treatment of rheumatoid arthritis with penicillamine and gold thiomalate the same? Proc. R. Soc. Med. 70 (Suppl. 3), 136–139.

Jellum, E., Munthe, E., Guldal, G., Aaseth, J., 1979. Gold and thiol compounds in the treatment of rheumatoid arthritis: excretory fate and tissue distribution of thiomalate in relation to gold after administration of myocrisin (aurothiomalate). Scand. J. Rheumatol., Suppl. 28, 28–36.

Jokay, I., Kelemenics, K., Gyuris, A., Minarovits, J., 1998. *S*-methylthio-cysteine and cystamine are potent stimulators of thiol production and glutathione synthesis. Life Sci. 62, PL27–PL33.

Kumar, K.S., Dobbs, C.R., Weiss, J.F., Chirigos, M.A., 1980. Levamisole inhibition of microsomal lipid peroxidation as related to its sulfhydryl metabolite DL-2-oxo-3-(2-mercaptoethyl)-5-phenylimadazolidine. J. Immunopharmacol. 2, 73–83.

Martin, M.F., Surrall, K.E., McKenna, F., Dixon, J.S., Bird, H.A., Wright, V., 1984. Captopril: a new treatment for rheumatoid arthritis? Lancet 1, 1325–1328.

Mazor, D., Greenberg, L., Shamir, D., Meyerstein, D., Meyerstein, N., 2006. Antioxidant properties of bucillamine: possible mode of action. Biochem. Biophys. Res. Commun. 349, 1171–1175.

McNutt-Scott, T.L., Harris, C., 1998. Modulation of intracellular glutathione and cysteine metabolism in bovine oviduct epithelial cells cultured In. vitro. Biol. Reprod. 59, 314–320.

Morquette, B., Shi, Q., Lavigne, P., Ranger, P., Fernandes, J.C., Benderdour, M., 2006. Production of lipid peroxidation products in osteoarthritic tissues: new evidence linking 4-hydroxynonenal to cartilage degradation. Arthritis Rheum. 54, 271–278.

Nagasawa, H.T., Goon, D.J., DeMaster, E.G., 1978. 2,5,5-Trimethylthiazolidine-4-carboxylic acid, a D(−)-penicillamine-directed pseudometabolite of ethanol. Detoxication mechanism for acetaldehyde. J. Med. Chem. 21, 1274–1279.

Peristeris, P., Clark, B.D., Gatti, S., Faggioni, R., Mantovani, A., Mengozzi, M., Orencole, S.F., Sironi, M., Ghezzi, P., 1992. *N*-acetylcysteine and glutathione as inhibitors of tumor necrosis factor production. Cell. Immunol. 140, 390–399.

Rudge, S.R., Perrett, D., Swannell, A.J., 1984. Free thiomalate levels in patients with rheumatoid arthritis treated with disodium aurothiomalate: relationship to clinical outcome of therapy. Ann. Rheum. Dis. 43, 698–702.

Seiler, N., 2004. Catabolism of polyamines. Amino Acids. 26, 217–233.

Tiku, M.L., Allison, G.T., Naik, K., Karry, S.K., 2003. Malondialdehyde oxidation of cartilage collagen by chondrocytes. Osteoarthr. Cartil. 11, 159–166.

Yokota, N., Kuga, Y., Kanazawa, T., Morishita, M., Tanida, K., Itou, K., 2007. Ten years results of bucillamine in the treatment of rheumatoid arthritis. Mod. Rheumatol. 17, 33–36.

Wade, C.R., Jackson, P.G., Highton, J., van Rij, A.M., 1987. Lipid peroxidation and malondialdehyde in the synovial fluid and plasma of patients with rheumatoid arthritis. Clin. Chim. Acta. 164, 245–250.

Wielandt, A.M., Vollrath, V., Farias, M., Chianale, J., 2006. Bucillamine induces glutathione biosynthesis via activation of the transcription factor Nrf2. Biochem. Pharmacol. 72, 455–462.

Wondrak, G.T., Cervantes-Laurean, D., Roberts, M.J., Qasem, J.G., Kim, M., Jacobson, E.L., Jacobson, M.K., 2002. Identification of alpha-dicarbonyl scavengers for cellular protection against carbonyl stress. Biochem. Pharmacol. 63, 361–673.

Wood, P.L., Cheney, D.L., 1985. Gas chromatography–mass fragmentography of amino acids. Neuromethods 3, 51–80.

Wood, P.L., Khan, M.A., Moskal, J.R., Todd, K.G., Tanay, V.A., Baker, G., 2006a. Aldehyde load in ischemia–reperfusion brain injury: neuroprotection by neutralization of reactive aldehydes with phenelzine. Brain. Res. 1122, 184–190.

Wood, P.L., Khan, M.A., Moskal, J.R., 2006b. Neurochemical analysis of amino acids, polyamines and carboxylic acids: GC-MS quantitation of tBDMS derivatives using ammonia positive chemical ionization. J. Chromatogr. B 831, 313–319.

Wood, P.L., Khan, M.A., Moskal, J.R., 2007a. The concept of "aldehyde load" in neurodegenerative mechanisms: cytotoxicity of the polyamine degradation products hydrogen peroxide, acrolein, 3-aminopropanal, 3-acetamidopropanal and 4-aminobutanal in a retinal ganglion cell line. Brain Res. 1145, 150–156.

Wood, P.L., Khan, A.K., Moskal, J.K., 2007b. Cellular thiol pools are responsible for sequestration of cytotoxic reactive aldehydes: Central role of free cysteamine and cysteine. Brain Res. 1158, 158–163.

Yu, Z., Li, W., Brunk, U.T., 2003. 3-Aminopropanal is a lysosomotropic aldehyde that causes oxidative stress and apoptosis by rupturing lysosomes. APMIS. 111, 643–652.

Yukioka, K., Wakitani, S., Yukioka, M., Furumitsu, Y., Shichikawa, K., Ochi, T., Goto, H., Matsui-Yuasa, I., Otani, S., Nishizawa, Y., 1992. Polyamine levels in synovial tissues and synovial fluids of patients with rheumatoid arthritis. J. Rheumatol. 19, 689–962.

Zeng, J., Davies, M.J., 2006. Protein and low molecular mass thiols as targets and inhibitors of glycation reactions. Chem. Res. Toxicol. 19, 1668–1676.