Amino Acids (1995) 8: 113–124

Amino
Acids

Stereoisomers of cysteine and its analogs Potential effects on chemo- and radioprotection strategies

Review Article

J. C. Roberts

Department of Medicinal Chemistry, University of Utah, Salt Lake City, Utah, U.S.A.

Accepted November 3, 1994

Summary. The thiol-containing amino acid, cysteine, and its analogs are useful for a variety of protective applications, including protecting normal tissues against the unwanted side effects of cancer chemotherapeutic agents and radiation treatment. The protection can result from both direct action of the amino acid and/or after its conversion to glutathione (GSH), sulfate, or other sulfur-based protective substances. Unfortunately, high GSH levels have been implicated in the problematic development of tumor cells' resistance to therapy. Due to numerous differences in the metabolic processing of the cysteine stereoisomers, chemo- and radioprotective strategies might be developed using the D-form of the amino acid, which can participate in protection directly, but which cannot be used to support GSH biosynthesis. In this way, protection of normal tissue may be achieved, while the potential development of resistance in tumor cells is minimized. Greatly enhanced therapeutic efficacy of cancer treatment regimens may be the result.

Keywords: Amino acids – Chemoprotection – Radioprotection – Cysteine – Glutathione – Stereospecificity

Cysteine has been widely studied for its ability to achieve both chemo- and radioprotection of cells, tissues, and organisms against a wide variety of toxic agents and situations. The thiol-containing amino acid can act directly or via conversion to glutathione (GSH), sulfate, or other sulfur-based protective species.

Numerous situations exist where protection strategies are desired or required. In the case of cancer treatment, protection of normal tissues against the unwanted, dose-limiting toxicity of cancer chemotherapeutic agents or radiation therapy would allow more efficacious treatment of a tumor. A major criticism against using cysteine in such a protective strategy is that the amino acid may: (a) promote the biosynthesis of GSH in tumor tissue and, therefore,

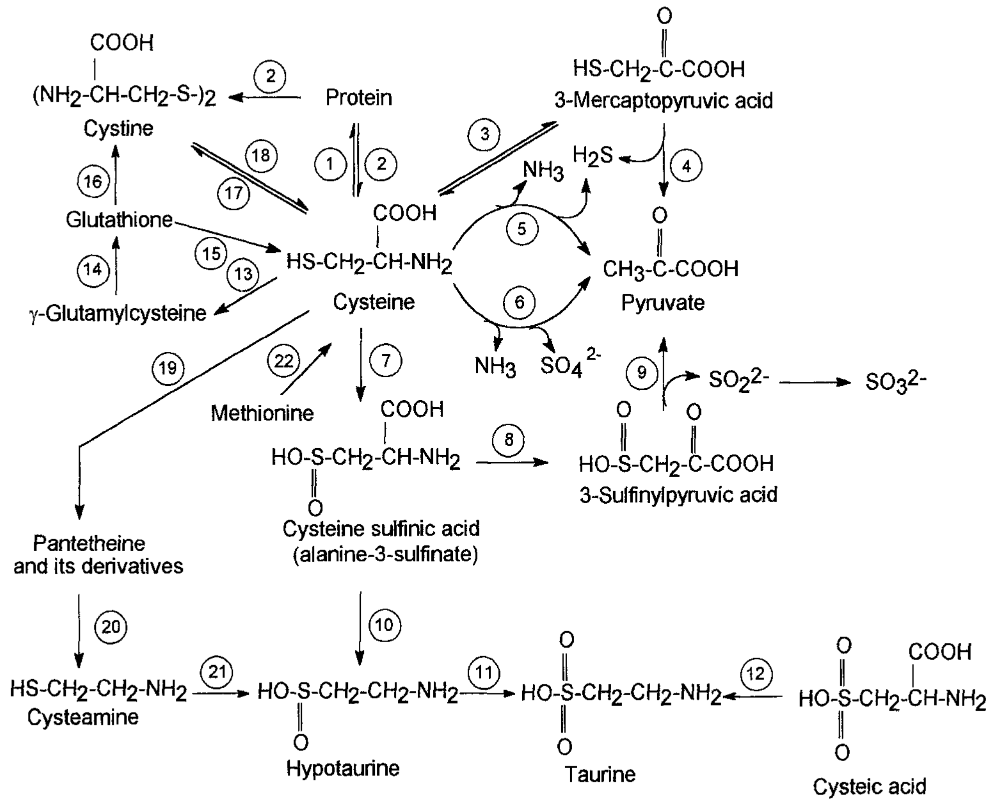

Fig. 1. Mammalian cysteine metabolism. The circled numbers correspond to the following enzymes or metabolic processes: *1*, ribosomal protein synthesis; *2*, formation of protein disulfide bonds; *3*, cysteine aminotransferase and probably other aminotransferases; *4*, 3-mercaptopyruvate sulfurtransferase; *5*, cystathionine γ-lyase; *6*, poorly characterized mitochondrial activity; *7*, cysteine dioxygenase; *8*, aspartate aminotransferase; *9*, spontaneous reaction; *10*, cysteinesulfinate decarboxylase; *11*, "hypotaurine oxidase"; *12*, cysteinesulfinate decarboxylase; *13*, γ-glutamylcysteine synthetase; *14*, glutathione synthase; *15*, combined activities of γ-glutamyltransferase and various dipeptidases; *16*, enzymatic and nonenzymatic oxidation of GSH to GSSG followed by cleavage reactions; *17*, various thiol oxidases; *18*, enzymatic and nonenzymatic transhydrogenations with GSH coupled to glutathione reductase; *19*, pantetheine and coenzyme A biosynthesis; *20*, pantetheine and coenzyme A catabolism; *21*, cysteamine dioxygenase; *22*, transsulfuration pathway. (From Griffith, 1987 with permission.)

promote resistance of the tumor to the drug treatment, and (b) achieve comparable protection in the tumor (DeLeve and Kaplowitz, 1991). Recent work (*vida infra*) with the two stereoisomers of cysteine has provided evidence that protection may be achieved against certain toxins (including chemotherapeutic agents) using the unnatural D-form of cysteine, which is incapable of supporting GSH biosynthesis and, therefore, incapable of promoting drug resistance in tumors. In addition, evidence exists that differential protection of normal tissue at the expense of tumor tissue can be achieved with protective compounds (Yuhas and Storer, 1969; Roberts et al., 1991). The differential protection is probably due to differences in blood flow between tumor and normal tissue and the potential active concentration of related agents by normal tissues (Yuhas, 1970). The hydrophilicity of protective agents also appears to be critical to the differential distribution (Brown et al., 1984).

Central to this issue is the stereospecific or stereoselective processing of cysteine. The complex metabolism of the L-amino acid is shown in Fig. 1 (Griffith, 1987). Two main metabolic routes have been recognized: (1) desulfhydration of cysteine to inorganic sulfate by a variety of enzyme systems commonly referred to as cysteine desulhydrases (cystathionine γ- and β-lyases and cysteine aminotransferase); and (2) oxidation of cysteine to cysteine sulfinate followed by conversion to taurine or pyruvate (Singer, 1975; Simpson and Freedland, 1976; Stipanuk, 1979; Horowitz et al., 1981; Stipanuk and Beck, 1982; Drake et al., 1987; Griffith, 1987; Hosokawa et al., 1988; Coloso et al., 1990). The balance between these two metabolic routes can be altered (Stipanuk et al., 1992). Production of GSH is another important pathway when considering implications to protection strategies.

Considerable study has been done particularly on the production of sulfate and taurine from cysteine stereoisomers and their oxidation products. Feeding L-cysteine to rats caused a dramatic increase in the urinary excretion of inorganic sulfate, cystine, taurine, and hypotaurine, but not of cysteic acid. D-Cysteine, on the other hand, was able to increase sulfate and cystine excretion to the same extent as the L-isomer, but no change in hypotaurine or taurine was observed (Cavallini et al., 1958; Krijgsheld et al., 1981; Glazenburg et al., 1984; Stipanuk and Rotter, 1984). Interestingly, the peak for serum sulfate was maximal by 30 minutes after D-cysteine administration, but required 2 hours to maximize after L-cysteine administration (Krijgsheld et al., 1981). The interpretation of these feeding studies is complicated by as yet undetermined potential differences between the two isomers that could effect the results. For example, the rate of uptake from the gut, the first pass metabolism in the liver, or the rate of sulfoxidation might be different. To avoid these problems, intravenous administration was utilized and resulted in very similar rates of plasma sulfate increase for the two stereoisomers (Glazenburg et al., 1984), in contrast to the data obtained after oral administration. While different paths to sulfate with identical rates could not be ruled out, these data suggested that the same enzyme system catalyzes the production of sulfate from both L- and D-cysteine. *In vitro*, the levels of L-cysteine appear to be critical in its metabolic conversions. High cysteine levels favor the production of sulfate and taurine, while low cysteine favors its incorporation into GSH (Meister, 1975; Stipanuk et al., 1992).

Sulfate production from D-cysteine may also arise by the action of D-amino acid oxidase, which produces 3-mercaptopyruvate. This enzyme has been found in the brain (Goldstein, 1966), kidney (Momoi et al., 1990; Krejci et al., 1991), liver (Schellens et al., 1992; Fahimi et al., 1993; Frederiks et al., 1993), retina (St. Jules et al., 1992), small intestine (Brachet and Puigserver, 1992; Cable et al., 1993), and normal and cancerous breast tissue (el Bouhtoury et al., 1992), primarily in the peroxisomes. The main role of this oxidase is thought to be the detoxication of D-amino acids before levels are reached that compromise the production of essential enzymes or other proteins or the normal growth of the organism (D'Aniello et al., 1993). The 3-

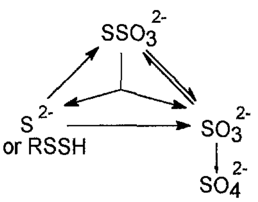

Fig. 2. Thiosulfate cycle (Stipanuk, 1979)

mercaptopyruvate produced by this enzyme may be transaminated to L-cysteine (Cooper et al., 1982), but this reaction seems unlikely because little or no taurine, a normal metabolite of the L-form, is observed after D-cysteine administration. Further metabolism of 3-mercaptopyruvate is more likely, producing pyruvate, thiosulfate, sulfide, or sulfate. These intermediates can go on to produce more sulfate through the "thiosulfate cycle" (Fig. 2, Sörbo, 1975; Stipanuk, 1979). Other products of 3-mercaptopyruvate have also been identified (Cooper et al., 1982). On the other hand, the similar toxicity of D- and L-S-(1,2-dichlorovinyl)cysteine has been associated with the D-amino acid oxidase-catalyzed conversion of the D- to the L-isomer (Wolfgang et al., 1989; Krejci et al., 1991).

Another possible route to sulfate from D-cysteine may be direct desufhydration. Bacterial and liver cysteine desulfhydrases have been shown to be non-stereospecific. In addition, the presence of both a D- and an L-cysteine desulfhydrase has been proposed for bacteria (Cavallini et al., 1958). However, Krijgsheld et al. (1981) state that desulfhydration of L-cysteine takes place by some as yet unidentified pathways, with little or no information available about the D-form.

The oxidation of cysteine to cysteine sulfinate is also of critical importance, in particular because sulfate can be an end product of this reaction as well. The stereospecificity of the process remains very controversial. Early work concluded that the enzyme involved, cysteine dioxygenase, was non-stereospecific (Cavallini et al., 1958). However, subsequent work has led to the conclusion that cysteine dioxygenase is highly specific for the L-form of the amino acid (Ewetz and Sörbo, 1966; Stipanuk, 1979; Krijgsheld et al., 1981). Glazenburg et al. (1984), on the other hand, believe that the stereospecificity observed in taurine production from L- and D-cysteine is not due to cysteine dioxygenase, but rather due to enzymes responsible for the subsequent decarboxylation to hypotaurine. These authors state, "The first step in the metabolic pathway from L-cysteine to both inorganic sulfate and taurine is catalyzed by the same enzyme, cysteine dioxygenase, leading to the first intermediate, alanine-3-sulfinate [cysteine sulfinate]. From this intermediate different pathways lead to sulfate or taurine." Stipanuk (1979), however, outlines many different pathways to sulfate (desulfhydration) that do not require prior oxidation to cysteine sulfinate, the common intermediate for taurine production. Because of these facts, interpretation of the Glazenburg information remains unclear.

When cysteic acid was fed to rats instead of cysteine, only the L-form was decarboxylated to taurine, confirming the belief that no known decarboxylase can utilize D-amino acids (Cavallini et al., 1958). Feeding cysteine sulfinate to rats resulted mainly in elevated sulfate levels in the case of the L-form, while the D-form increased sulfate to only a limited extent. Unexpectedly, feeding studies with D-cysteine sulfinate also resulted in the excretion of a relatively large amount of taurine (Cavallini et al., 1958). This may indicate the stereospecific deamination and reamination to the L-sulfinic acid, in contrast to the situation with cysteine itself. In general, Cavallini and co-workers (1958) concluded that production of sulfate is carried out by non-stereospecific processes. The conversion to taurine or hypotaurine, however, is catalyzed by stereospecific enzymes. In contrast, recent work has characterized D-cysteine sulfinate as an inhibitor of cysteine sulfinate decarboxylase (Weinstein and Griffith, 1987; Weinstein et al., 1988).

Other physiologically important differences between the cysteine stereoisomers have been studied as well. Differential uptake of L- and D-amino acids across the blood-brain barrier (Oldendorf, 1973), erythrocyte membrane (Winter and Christensen, 1964), and intestinal epithelia (Gibson and Wiseman, 1951) has been documented, although cysteine stereoisomers were not specifically studied in these cases. D-Amino acids cannot function as substrates for cysteine conjugate β-lyase (Tateishi and Shimizu, 1981). D-Cysteine was found to be a poor inhibitor of cysteine-specific lysosomal transport, indicating stereospecificity of the system (Pisoni et al., 1990). This unnatural isomer suppresses growth in mice, while L-cysteine supports normal function and development and demonstrates a sparing effect on L-methionine levels (Friedman and Gumbmann, 1984; Friedman, 1991). Interestingly, contamination of commercial preparations of L-amino acids, including L-cysteine, with the D-enantiomer has been reported (Armstrong et al., 1991). This could have interesting ramifications on any biochemical or therapeutic studies that have been conducted using these preparations.

L-Cysteine has been shown to be mutagenic in the Ames assay, under numerous metabolic conditions, and very toxic to neonatal rats (White et al., 1993). In contrast, D-cysteine did not exhibit measurable mutagenicity and, in fact, actually decreased the number of revertant colonies per plate from control levels. This fact was explained by the probable bacteriotoxicity of the D-enantiomer (Glatt and Oesch, 1985).

L-Cysteine is also a well known neurotoxin (Olney et al., 1990). Considerable evidence has pointed to the role of cysteine sulphinate and cysteic acid in this toxicity (Lehmann et al., 1993), further emphasizing the importance of the cysteine oxidative enzyme system. The excitotoxic response, primarily seen in the cerebral cortex, was eliminated by pretreatment with an N-methyl-D-aspartate (NMDA) receptor antagonist, MK-801. Other studies have also implicated glutamate receptors in the toxic action of L-cysteine, as well as the amino acid's effects on intracellular calcium, inositol phosphate, and cGMP levels (Frandsen et al., 1993; Porter and Roberts, 1993; Gorman and Griffiths, 1994). In hippocampal slices, both L-cysteine and L-cysteic acid produced profound toxicity in energy-deprived preparations, which was again blocked with NMDA receptor antagonists (Schurr et al., 1993). Both cysteine sulfinate and cysteic acid caused an enhancement of ethanol's depressant activity in the central nervous system in mice (Ferko, 1993). Using taurine receptor preparations from mammalian brain, cysteic acid was shown to be a powerful inhibitor of ^{3}H-taurine binding, the physiological significance of which is not understood (Wu et al., 1992). The effects of the D-form on these parameters has not been reported.

In other measures of toxicity, the administration of L-cysteine under certain conditions caused a decrease in hepatic GSH content (Viña et al., 1983). The observed effect was less pronounced when the amino acid was administered orally. This toxic effect of L-cysteine was attributed mainly to autoxidation of the amino acid. Such an oxidative process generates hydroxyl and thiyl radicals, which result in GSH depletion and other signs of cell damage, either directly or through the metal-catalyzed Fenton reaction from hydrogen peroxide (Held and Biaglow, 1993). Autoxidation is particularly pronounced after intravenous administration. If L-cysteine is administered orally, however, the gastric pH maintains the amino acid predominantly in its reduced form (Estrela et al., 1983). Oxidation and production of free radicals should be independent of configuration. Related information on the D-form is limited to the work of Dewey and Gaugas (1980) who reported similar toxicities between the two stereoisomers that could only be explained by similarities in chemical autoxidation rather than in any enzymatic process. The observed toxicity, in most cases, is more pronounced at low doses and disappears at higher, more physiological levels of cysteine. In addition, oxidative products of L-cysteine and dopamine conjugates that form in the brain showed profound toxicity *in vivo*, and have been linked to the progression of Parkinson's disease (Zhang and Dryhurst, 1994).

The interrelationship between cysteine and GSH can be very important in the context of protective experiments. It is known that D-cysteine readily enters hepatocytes, but it does not support GSH biosynthesis (Glazenburg et al., 1984; Wong and Corcoran, 1990). Acetaminophen hepatotoxicity has been widely used as a model for assessing requirements for protection. L-Cysteine exhibits protective capabilities against acetaminophen hepatotoxicity, but D-cysteine does not; therefore, GSH must be required for protection (Corcoran and Wong, 1986; McLean et al., 1989). An identical situation was seen using the two stereoisomers after 2-cyclohexene-1-one intoxication (Masukawa et al., 1989). These parameters have been extensively examined using N-acetylcysteine (NAC), a prodrug of cysteine that liberates the free amino acid after enzymatic deacylation. NAC is widely used as an antidote for acetaminophen overdose. Numerous mechanisms exist by which NAC could exhibit its protective effects. Studies using NAC synthesized from both L- and D-cysteine have demonstrated that the prodrug's protective abilities stem from the release of L-cysteine, which provides a critical precursor for GSH biosynthesis (Corcoran and Wong, 1986; Wong et al., 1986). Direct effects of the free sulfhydryl groups were ruled out. However, Burgunder et al. (1989) report that the levels of free cysteine seen after L-NAC administration are at least partly due to disulfide exchange with endogenous cystine, which liberates a molecule of L-cysteine. Such chemical reduction should occur to an equivalent degree for the two stereoisomers. In any case, the vast majority of free L-cysteine resulting from NAC is the result of enzymatic deacylation, a reaction which proceeds very slowly if at all with D-amino acids (Birnbaum et al., 1952). In similar studies, both isomers were found to be equipotent as antidotes to acrylonitrile intoxication (Benz et al., 1990).

The effect of L-NAC administration on hepatic GSH varies widely. Results outlining GSH increase, decrease, or no change have been reported following the administration of NAC (Estrela et al., 1983; Karg et al., 1990), depending upon the experimental protocol. GSH depletion by NAC has been linked to the production of the reactive radicals generated by autoxidation, similar to the situation with cysteine itself. D-NAC produces dose-dependent toxicity *in vitro* as did L-NAC, but to a much smaller extent than the L-enantiomer. The stereoisomers were not compared with respect to their extent of autoxidation. In addition, L-NAC, but not its D-isomer, significantly increased plasma and urine sulfate levels in mice (Wong et al., 1986).

Another prodrug of cysteine, 2-oxothiazolidine-4-carboxylate (OTZ), was synthesized with both L- and D-cysteine, and the properties of the two derivatives were compared (Williamson and Meister, 1981; Williamson and Meister, 1982). The L-isomer was found to be a good substrate for 5-oxo-L-prolinase, resulting in the release of L-cysteine from the prodrug. No reaction was observed when the substrate was replaced by the D-form. In addition, L-OTZ caused a 2-fold increase in hepatic GSH 4 hours after intraperitoneal administration; inorganic sulfur and taurine represented other metabolic products (Coloso et al., 1991). No such stimulation was observed when equivalent doses of the D-form of OTZ were administered. Increases in GSH production appear to be best achieved at low OTZ and, therefore, low L-cysteine concentrations, while high OTZ leads to increased taurine and sulfate instead of GSH (Stipanuk et al., 1992).

Similar results were obtained with another prodrug of cysteine, 2-methylthiazolidine-4-carboxylic acid (2-MTCA) (Nagasawa et al., 1984; Hazelton et al., 1986). The L-form protected against acetaminophen's hepatotoxicity while the D-isomer was completely without protective abilities, confirming the requirement for GSH in acetaminophen detoxication.

Other thiazolidine prodrugs of cysteine have been synthesized using aldose monosaccharides as the carbonyl donor for heterocyclic ring formation (Roberts et al., 1986). Of the eight analogs tested, the prodrug constructed from L-cysteine and D-ribose (RibCys) showed the greatest ability to protect against acetaminophen-induced hepatotoxicity (Roberts et al., 1986; Roberts et al., 1992). In addition, RibCys was shown to be an effective chemoprotective agent against cyclophosphamide-induced toxicity to the bladder (production of dose-limiting hemorrhagic cystitis) without compromising the anticancer activity of the drug (Roberts et al., 1991; Roberts and Francetic, 1994). Further studies in the area included the preparation of RibCys using D-cysteine (D-RibCys), which, along with D-cysteine itself, showed equal or slightly better uroprotective activity than their L-analogs (Roberts et al., 1994). These results are in direct contrast to those found for protection against acetaminophen's hepatotoxicity and suggest that GSH cannot be required for detoxication in this case.

Summary

The L- and D-forms of cysteine are converted to inorganic sulfate to a very similar extent, but only the L-isomer is capable of producing taurine as a metabolite or of being incorporated into GSH. The importance of the many pathways available to cysteine for sulfate production is not clear. It is important to determine which enzymes in the pathways exhibit stereospecificity toward their substrate in order to sort out potential effects of cysteine stereoisomers in therapeutic applications such as chemo- and radioprotection. If cysteine dioxygenase only accepts L-cysteine, then the other less well understood pathways to sulfate, not passing through cysteine sulfinate, are major ones for the D-form. In this scenario, the conversion to hypotaurine/taurine may or may not exhibit stereospecificity. If cysteine dioxygenase is not stereospecific, more pathways are available to D-cysteine for the production of sulfate. In this scenario, the conversion of cysteine sulfinate to hypotaurine must be stereospecific.

With respect to protection experiments, numerous interesting points emerge. One possible explanation for the protective effects of D-cysteine and D-RibCys against cyclophosphamide urotoxicity is the conversion of the amino acid to 3-mercaptopyruvate. Apparently this transformation can be carried out by D-amino acid oxidase; little information is available about the properties of cysteine aminotransferase, which is also capable of carrying out the reaction (Friedman and Gumbmann, 1984). Transamination of this intermediate produces L-cysteine, which could be responsible for the protective effects. However, as stated above, other products of L-cysteine, namely taurine, are not observed after D-cysteine administration, making this possibility unlikely.

Taken together, literature information, as well as recent experimental data, suggests that protection against cyclophosphamide urotoxicity proceeds by a different mechanism than does protection against acetaminophen hepatotoxicity. Without question, GSH is a required agent in acetaminophen detoxication, and D-cysteine is incapable of protection because it cannot support GSH biosynthesis. However, in the case of cyclophosphamide, direct detoxication of the toxic metabolite, acrolein, by sulfhydryl-containing compounds, perhaps in the urine itself, appears to be the major route of protection. This may explain the similar abilities of D- and L-cysteine and D- and L-RibCys to protect the bladder. Accessibility of the intact amino acids of either configuration to the bladder is an unresolved issue, although extensive urinary excretion is likely in both cases. While this may be a fortuitous combination of circumstances unique to cyclophosphamide, further study with other chemotherapeutic agents is warranted.

Choosing a protection strategy particularly for a cancer treatment situation will depend on many things, including the nature of the therapeutic agent and its mechanisms of clearance. For those substances not requiring GSH to mediate detoxication, using the D-isomer of cysteine or its analogs in chemoprotection offers a superior approach because these compounds are incapable of supporting GSH biosynthesis in the tumor cells, a phenomenon linked to the development of resistance to treatment. Increased efficacy of cancer treatment regimens may be the result.

References

Armstrong DW, Duncan JD, Lee S (1991) Evaluation of D-amino acid levels in human urine and in commercial L-amino acid samples. Amino Acids 1: 97–106

Benz FW, Nerland DE, Pierce WM, Babiuk C (1990) Acute acrylonitrile toxicity: studies on the mechanism of the antidotal effect of D- and L-cysteine and their N-acetyl derivatives in the rat. Toxicol Appl Pharmacol 102: 142–150

Birnbaum SM, Levintow J, Kingsley RB, Greenstein JP (1952) Specificity of amino acid acylases. J Biol Chem 194: 455–470

Brachet P, Puigserver A (1992) Regional differences for the D-amino acid oxidase-catalysed oxidation of D-methionine in chicken small intestine. Comp Biochem Physiol B 101: 509–511

Brown DQ, Yuhas JM, MacKenzie LJ, Graham WJ, Pittock JW (1984) Differential radioprotection of normal tissues by hydrophilic chemical protectors. Int J Radiat Oncol Biol Phys 10: 1581–1584

Burgunder JM, Varriale A, Lauterburg BH (1989) Effect of N-acetylcysteine on plasma cysteine and glutathione following paracetamol administration. Eur J Clin Pharmacol 36: 127–131

Cable S, Kedinger M, Dauca M (1993) Peroxisomes and peroxisomal enzymes along the crypt-villus axis of the rat intestine. Differentiation 54: 99–108

Cavallini D, De Marco C, Mondovi B (1958) Experiments with D-cysteine in the rat. J Biol Chem 239: 25–30

Coloso RM, Drake MR, Stipanuk MH (1990) Effect of bathocuproine disulfonate, a copper chelator, on cyst(e)ine metabolism by freshly isolated rat hepatocytes. Am J Physiol 259: E443–E450

Coloso RM, Hirschberger LL, Stipanuk MH (1991) Uptake and metabolism of L-2-oxo-[^{35}S]thiazolidine-4-carboxylate by rat cells is slower than that of L-[^{35}S]cysteine or L-[^{35}S]methionine. J Nutr 121: 1341–1348

Cooper AJL, Haber MT, Meister A (1982) On the chemistry and biochemistry of 3-mercaptopyruvic acid, the α-keto acid analog of cysteine. J Biol Chem 257: 816–826

Corcoran GB, Wong BK (1986) Role of glutathione in prevention of acetaminophen-induced hepatotoxicity by N-acetyl-L-cysteine *in vivo*: studies with N-acetyl-D-cysteine in mice. J Pharmacol Exper Ther 238: 54–61

D'Aniello A, D'Onofrio G, Pischetola M, D'Aniello G, Vetere A, Putrucelli L, Fisher GH (1993) Biological role of D-amino acid oxidase and D-aspartate oxidase. Effects of D-amino acids. J Biol Chem 268: 26941–26949

DeLeve LD, Kaplowitz N (1991) Glutathione metabolism and its role in hepatotoxicity. Pharmacol Ther 52: 287–305

Dewey DL, Gaugas JM (1980) Inhibition of proliferation of cultured cells by polyamine and cysteine interaction. Cancer Letts 10: 229–234

Drake MR, De La Rosa J, Stipanuk MH (1987) Metabolism of cysteine in hepatocytes. Evidence for cysteinesulphinate-independent pathways. Biochem J 244: 279–286

el Bouhtoury F, Keller JM, Colin S, Parache R, Dauca M (1992) Peroxisomal enzymes in normal and tumoral human breast. J Pathol 166: 27–35

Estrela JM, Sáez G, Such L, Viña J (1983) The effect of cysteine and N-acetyl cysteine on rat liver glutathione (GSH). Biochem Pharmacol 32: 3483–3485

Ewetz L, Sörbo B (1966) Characteristics of the cysteinesulfinate-forming enzyme system in rat liver. Biochim Biophys Acta 128: 296–305

Fahimi HD, Baumgart E, Volkl A (1993) Ultrastructural aspects of the biogenesis of peroxisomes in rat liver. Biochimie 75: 201–208

Ferko AP (1993) Cysteine sulfinic acid can enhance the central depressant effect of ethanol in mice. Pharmacol Biochem Behav 39: 653–657

Frandsen A, Schousboe A, Griffiths R (1993) Cytotoxic actions and effects on intracellular Ca^{2+} and cGMP concentrations of sulphur-containing excitatory amino acids in cultured cerebral cortical neurons. J Neurosci Res 34: 331–339

Frederiks WM, Van Noorden CJ, Marx F, Gallagher PT, Swann BP (1993) In situ kinetic measurements of D-amino acid oxidase in rat liver with respect to its substrate specificity. Histochem J 25: 578–582

Friedman M (1991) Formation, nutritional value, and safety of D-amino acids. In: Friedman M (ed) Nutritional and toxicological consequences of food processing. Plenum Press, New York, pp 447–481

Friedman M, Gumbmann MR (1984) The utilization and safety of isomeric sulfur-containing amino acids in mice. J Nutr 114: 2301–2310

Gibson QJ, Wiseman G (1951) Selective absorption of stereo-isomers of amino-acids from loops of the small intestine of the rat. Biochem J 48: 426–429

Glatt H, Oesch F (1985) Mutagenicity of cysteine and penicillamine and its enantiomeric selectivity. Biochem Pharmacol 34: 3725–3728

Glazenburg EJ, Jekel-Halsema IMC, Baranczyk-Kuzma A, Krijgsheld KR, Mulder GJ (1984) D-Cysteine as a selective precursor for inorganic sulfate in the rat in vivo. Effect of D-cysteine on the sulfation of harmol. Biochem Pharmacol 33: 625–628

Goldstein DB (1966) D-Amino acid oxidase in brain: distribution in several species and inhibition by pentobarbitone. J Neurochem 13: 1011–1016

Gorman A, Griffiths R (1994) Sulphur-containing excitatory amino acid-stimulated inositol phosphate formation in primary cultures of cerebellar granule cells in mediated predominantly by N-methyl-D-aspartate receptors. Neuroscience 59: 299–308

Griffith OW (1987) Mammalian sulfur amino acid metabolism: An overview. Methods Enzymol 143: 366–376

Hazelton GA, Hjelle JJ, Klaassen CD (1986) Effects of cysteine pro-drugs on acetaminophen-induced hepatotoxicity. J Pharmacol Exp Ther 237: 341–349

Held KD, Biaglow JE (1993) Role of copper in the oxygen radical-mediated toxicity of the thiol-containing radioprotector dithiothreitol in mammalian cells. Radiat Res 134: 375–382

Horowitz JH, Rypins EB, Henderson JM, Heymsfield SB, Moffitt ST, Bain RP, Chawla RK, Bleier JC, Rudman D (1981) Evidence for impairment of transsulfuration pathway in cirrhosis. Gastroenterol 81: 668–675

Hosokawa Y, Niizeki S, Tojo H, Sato I, Yamaguchi K (1988) Hepatic cysteine dioxygenase activity and sulfur amino acid metabolism in rats: possible indicators in the evaluation of protein quality. J Nutr 118: 456–461

Karg E, Tunek A, Brötell H, Rosengren E, Rorsman H (1990) Alteration of glutathione level in human melanoma cells: effect of N-acetyl-L-cysteine and its analogues. Pigment Cell Res 3: 11–15

Krejci ME, Ridgewell RE, Koechel DA (1991) Acute effects of the D-isomer of S-(1,2-dichlorovinyl)cysteine on renal function and ultrastructure in the pentobarbital-anesthetized dog: site-specific toxicity involving the S_1 and S_2 cells of the proximal tubule. Toxicology 69: 151–164

Krijgsheld KR, Glazenburg EJ, Scholtens E, Mulder GJ (1981) The oxidation of L- and D-cysteine to inorganic sulfate and taurine in the rat. Biochim Biophys Acta 677: 7–12

Lehmann A, Hagberg H, Orwar O, Sandberg M (1993) Cysteine sulphinate and cysteate: mediators of cysteine toxicity in the neonatal rat brain? Eur J Neurosci 5: 1398–1412

Masukawa T, Sai M, Tochino Y (1989) Brain glutathione and the anti-hypoxic effect of glutathione depletors in mice. Jpn J Pharmacol 51: 125–127

McLean AE, Armstrong GR, Beales D (1989) Effect of D- or L-methionine and cysteine on the growth inhibitory effects of feeding 1% paracetamol to rats. Biochem Pharmacol 38: 347–352

Meister A (1975) Biochemistry of glutathione. In: Greenberg DM (ed) Metabolism of sulfur compounds. Academic Press, New York, pp 101–188

Momoi K, Fukui K, Tada M, Miyake Y (1990) Gene expression of D-amino acid oxidase in rabbit kidney. J Biochem Tokyo 108: 406–413

Nagasawa HT, Goon DJW, Muldoon WP, Zera RT (1984) 2-Substituted thiazolidine-4(R)-carboxylic acids as prodrugs of L-cysteine. Protection of mice against acetaminophen hepatotoxicity. J Med Chem 27: 591–596

Oldendorf WH (1973) Stereospecificity of blood-brain barrier permeability to amino acids. Am J Physiol 224: 967–969

Olney JW, Zorumski C, Price MT, Labruyere J (1990) L-Cysteine, a bicarbonate-sensitive endogenous excitotoxin. Science 240: 596–599

Pisoni RL, Acker TL, Lisowski KM, Lemons RM, Thoene JG (1990) A cysteine-specific lysosomal transport system provides a major route for the delivery of thiol to human fibroblast lysosomes: possible role in supporting lysosomal proteolysis. J Cell Biol 110: 327–335

Porter RH, Roberts PJ (1993) Glutamate metabotropic receptor activation in neonatal rat cerebral cortex by sulphur-containing excitatory amino acids. Neurosci Lett 154: 78–80

Roberts JC, Francetic DJ (1994) Chemoprotection against cyclophosphamide-induced urotoxicity: Ribose-Cysteine. Anticancer Res 14: 383–388

Roberts JC, Nagasawa HT, Zera RT, Fricke RF, Goon DJW (1986) Prodrugs of L-cysteine as protective agents against acetaminophen-induced hepatotoxicity. 2-(Polyhydroxyalkyl)- and 2-(polyacetoxyalkyl)thiazolidine-4(R)-carboxylic acids. J Med Chem 30: 1891–1896

Roberts JC, Francetic DJ, Zera RT (1991) L-Cysteine prodrug protects against cyclophosphamide urotoxicity without compromising therapeutic activity. Cancer Chemother Pharmacol 28: 389–396

Roberts JC, Charyulu RL, Francetic DJ, Zera RT, Nagasawa HT (1992) Protection against acetaminophen hepatotoxicity by Ribose-Cysteine (RibCys). Pharmacol Toxicol 70: 281–285

Roberts JC, Francetic DJ, Zera RT (1994) Chemoprotection against cyclophosphamide-induced urotoxicity: comparison of nine thiol protective agents. Anticancer Res 14: 389–395

Schellens JP, Frederiks WM, Van Noorden CJ, Vreeling-Sindelarova H, Marx F, McMillan PJ (1992) The use of unfixed cryostat sections for electron microscopic study of D-amino acid oxidase activity in rat liver. J Histochem Cytochem 40: 1975–1979

Schurr A, West CA, Heine MF, Rigor BM (1993) The neurotoxicity of sulfur-containing amino acids in energy-deprived rat hippocampal slices. Brain Res 601: 317–320

Simpson RC, Freedland RA (1976) Factors affecting the rate of gluconeo genesis from L-cysteine in the perfused rat liver. J Nutr 106: 1272–1278

Singer TP (1975) Oxidative metabolism of cysteine and cystine in animal tissues. In: Greenberg DM (ed) Metabolism of sulfur compounds. Academic Press, New York, pp 535–546

Sörbo B (1975) Thiosulfate sulfurtransferase and mercaptopyruvate sulfurtransferase. In: Greenberg DM (ed) Metabolism of sulfur compounds. Academic Press, New York, pp 433–456

Stipanuk MH (1979) Effect of excess dietary methionine on the catabolism of cysteine in rats. J Nutr 109: 2126–2139

Stipanuk MH, Beck PW (1982) Characterization of the enzymatic capacity for cysteine desulphhydration in liver and kidney of the rat. Biochem J 206: 267–277

Stipanuk MH, Rotter MA (1984) Metabolism of cysteine, cysteinesulfinate and cysteinesulfonate in rats fed adequate and excess levels of sulfur-containing amino acids. J Nutr 114: 1426–1437

Stipanuk MH, Coloso RM, Garcia RAG, Banks MF (1992) Cysteine concentration regulates cysteine metabolism to glutathione, sulfate and taurine in rat hepatocytes. J Nutr 122: 420–427

St. Jules R, Kennard J, Stelik W, Holtzman E (1992) Frog cones as well as Muller cells have peroxisomes. Exp Eye Res 54: 1–8

Tateishi M, Shimizu H (1981) Cysteine conjugate β-lyase. Methods Enzymol 77: 253–264

Viña J, Saez GT, Wiggins D, Roberts AFC, Hems R, Krebs H (1983) The effect of cysteine oxidation on isolated hepatocytes. Biochem J 212: 39–44

Weinstein CL, Griffith OW (1987) Multiple forms of rat liver cysteinesulfinate decarboxylase. J Biol Chem 262: 7254–7263

Weinstein CL, Haschemeyer RH, Griffith OW (1988) In vivo studies of cysteine metabolism. Use of D-cysteinesulfinate, a novel cysteinesulfinate decarboxylase inhibitor, to probe taurine and pyruvate synthesis. J Biol Chem 263: 16568–16579

White RD, Wilson DM, Glosson JA, Madsen DC, Rowe WB, Goldberg DI (1993) Toxicity evaluations of L-cysteine and Procysteine™, a cysteine prodrug, given once intravenously to neonatal rats. Toxicology Letts 69: 15–24

Williamson JM, Meister A (1981) Stimulation of hepatic glutathione formation by administration of L-2-oxothiazolidine-4-carboxylate, a 5-oxo-L-prolinase substrate. Proc Natl Acad Sci 78: 936–939

Williamson JM, Meister A (1982) New substrates of 5-oxo-L-prolinase. J Biol Chem 257: 12039–12042

Winter CG, Christensen HN (1964) Migration of amino acids across the membrane of the human erythrocyte. J Biol Chem 239: 872–878

Wolfgang GH, Gandolfi AJ, Stevens JL, Brendel K (1989) In vitro and in vivo nephrotoxicity of the L and D isomers of S-(1,2-dichlorovinyl)-cysteine. Toxicology 58: 33–42

Wong BK, Corcoran GB (1990) N-Acetylcysteine stereoisomers as *in vivo* probes of the role of glutathione in drug detoxification. In: Viña J (ed) Glutathione: metabolism and physiological functions. CRC Press, Boston, pp 255–262

Wong BK, Chan HC, Corcoran GB (1986) Selective effects of N-acetylcysteine stereoisomers on hepatic glutathione and plasma sulfate in mice. Toxicol Appl Pharmacol 86: 421–429

Wu JY, Tang XW, Tsai WJ (1992) Taurine receptor: kinetic analysis and pharmacological studies. Adv Exp Med Biol 315: 263–268

Yuhas JM (1970) Biological factors affecting the radioprotective efficiency of S-2-(3-aminopropylamine)ethyl phosphorothioic acid (WR 2721). $LD_{50/30}$ doses. Radiat Res 44: 621–628

Yuhas JM, Storer JB (1969) Differential chemoprotection of normal and malignant tissues. J Natl Cancer Inst 42: 331–335

Zhang F, Dryhurst G (1994) Effects of L-cysteine on the oxidation chemistry of dopamine: new reaction pathways of potential relevance to idiopathic Parkinson's disease. J Med Chem 37: 1084–1098

Author's address: Dr. J. C. Roberts, Department of Medicinal Chemistry, 308 Skaggs Hall, University of Utah, Salt Lake City, UT 84112, U.S.A.

Received September 5, 1994