Metabolism of the Tumor Angiogenesis Inhibitor 4-(N-(S-Glutathionylacetyl)amino)phenylarsonous Acid*

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4-(N-(S-glutathionylacetyl)amino) phenylarsonous acid (GSAO) is a small, synthetic mitochondrial poison that targets angiogenic endothelial cells and is currently being tested in a Phase I/IIa clinical trial. The trivalent arsenical of GSAO reacts with and perturbs adenine nucleotide translocase of the inner mitochondrial membrane of endothelial cells, which leads to proliferation arrest. Three observations indicated that the γ-glutamyl residue of GSAO is cleaved at the endothelial cell surface by γ-glutamyl transpeptidase (γGT). GSAO was found to be an efficient substrate for γGT, endothelial cell accumulation and antiproliferative activity of GSAO was blunted by a competitive substrate and an active site inhibitor of γGT, and the level of cell surface γGT correlated strongly with the sensitivity of cells to GSAO. Using transport inhibitors, it was revealed that the resulting metabolite of GSAO cleavage by γGT, 4-(N-(S-cysteinylglycylacetyl)amino)phenylarsonous acid (GCAO), was transported across the plasma membrane by an organic anion transporter. Furthermore, GCAO is likely processed by dipeptidases in the cytosol to 4-(N-(S-cysteinylacetyl)amino) phenylarsonous acid (CAO), and it is this metabolite that reacts with mitochondrial adenine nucleotide translocase. Taken together, our findings indicate that γGT processing of GSAO at the cell surface is the rate-limiting step in its antiangiogenic activity. This information can explain the kidney toxicity at high doses of GSAO noted in preclinical studies and will aid in the anticipation of potential side effects in humans and in the design of better antimitochondrial cancer drugs.

GSAO is a mitochondrial poison that selectively perturbs angiogenic endothelial cells in vitro and in vivo (1–3). The tripeptide trivalent arsenical inactivates the mitochondrial inner membrane transporter, adenine nucleotide translocase (ANT), by cross-linking two of the three matrix facing cysteine thiols (1, 4). Proper functioning of ANT is essential for cell viability, so targeting this protein in angiogenic endothelial cells is a powerful means of blocking angiogenesis (5). A limitation of targeting specific angiogenic proteins is that they can often be circumvented by other proteins in the angiogenic process. GSAO is currently being tested in a Phase I/IIa clinical trial in cancer patients.

ANT exchanges matrix ATP for intermembrane space ADP across the inner mitochondrial membrane and is a key component of the mitochondrial permeability transition pore (6, 7). Inactivation of ANT by GSAO causes an increase in superoxide levels, proliferation arrest, ATP depletion, mitochondrial depolarization, and apoptosis in endothelial cells. The strong selectivity of GSAO for proliferating endothelial cells is a consequence of the higher mitochondrial calcium levels in proliferating cells (1). ANT is a calcium receptor that undergoes a conformational change and a change in activity upon binding of calcium ions. GSAO binds to calcium-replete ANT but binds minimally in the absence of calcium ions. Calcium content within the mitochondrial matrix increases severalfold in proliferating cells, and it is the higher mitochondrial calcium concentration that sensitizes proliferating cells to GSAO-induced pore formation. This mechanism is supported by the crystal structure of the calcium-free form of bovine ANT (8) (Protein Data Bank ID 1OKC). The members of the cysteine pair (Cys-160 and Cys-257) that GSAO is proposed to cross-link in the calcium-replete form are 18 Å apart in the calcium-free structure, which is at least twice the distance required for them to interact with trivalent arsenicals (9).

GSAO is also a selective inhibitor of endothelial cells when compared with tumor cells. For instance, GSAO is >30 times more effective at inhibiting proliferation of endothelial cells than human pancreatic Bx-PC3 carcinoma cells. This is because endothelial cells are very poor at exporting GSAO, whereas the multidrug resistance-associated proteins (MRP) 1 and 2 in tumor and other cells efficiently pump GSAO out the cytoplasm (2). Endothelial cells express very little MRP1/2.

This study addressed the question of how GSAO translocates across the plasma membrane of endothelial cells. We hypothesized that the glutathione pendant is the moiety that mediates the entry of GSAO into cells. There are no efficient plasma membrane transporters of glutathione in mammalian cells, however. Considering that GSAO resembles a glutathione-S

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* This study was supported by grants from the National Health and Medical Research Council of Australia, the Cancer Council New South Wales (NSW), the Cancer Institute NSW and the Istituto Toscano Tumori (Firenze, Italy). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

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§ The abbreviations used are: GSAO, 4-(N-(S-glutathionylacetyl)amino)phenylarsonous acid; CAO, 4-(N-(S-cysteinylacetyl)amino)phenylarsonous acid; GCAO, 4-(N-(S-cysteinylglycylacetyl)amino)phenylarsonous acid; DIDS, 4,4′-disothiocyanostilbene-2,2′-disulfonic acid; OATP, organic anion-transporting polypeptide; γGT, γ-glutamyl transpeptidase; ANT, adenine nucleotide translocase; MRP, multidrug resistance-associated proteins; BAE, bovine aortic endothelial; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; ABBA, L-2-amino-4-borobutanoic acid; HPLC, high pressure liquid chromatography.
conjugate of aminophenylarsonous acid, we explored the possibility that GSAO was metabolized at the cell surface and that a product containing the arsenical moiety was transported across the plasma membrane.

We show here that the γ-glutamyl residue of GSAO is cleaved from the molecule at the cell surface by γ-glutamyltransferase (γGT; EC 2.3.2.2) to produce 4-(N-(S-cysteinylglycylacetyl)amino) phenylarsonous acid (GCAO). GCAO in then transported across the plasma membrane by the organic anion-transporting polypeptide family. These findings indicate that GSAO could be considered a pro-drug that is dependent on cell surface processing by γGT for its action.

**EXPERIMENTAL PROCEDURES**

**Cell Proliferation and Viability Assays**—Bovine aortic endothelial (BAE) cells were from Cell Applications (San Diego, CA). BAE cells were cultured in Dulbecco’s modified Eagle’s medium supplemented with 10% fetal calf serum, 2 mM l-glutamine, and 5 units/ml penicillin and streptomycin (Invitrogen). Melanoma cell clones expressing different γGT activity were produced as described previously (10). The c21/basal and c21/γGT clones express a γGT activity of 0.34 ± 0.13 and 91 ± 3.4 million units/mg of cellular protein, respectively. Both clones were grown in RPMI 1640 medium, supplemented with 10% (v/v) heat-inactivated fetal calf serum, 2 mM l-glutamine, and 0.5 mg/ml G418 (Invitrogen). Cells were cultured at 37 °C in a 5% CO₂, 95% air atmosphere. Cell culture plasticware was from Techno Plastic Products (Trasadingen, Switzerland). All other cell culture reagents were from Invitrogen.

BAE (5,000 cells/well) and melanoma cells (4,000 cells/well) were seeded in 96-well plates in 0.2 ml of culture medium. Viable BAE cells were determined using the WST-1 reagent (Roche Diagnostics) according to the manufacturer’s protocol. Results were expressed as the percentage of untreated controls. The extinction of cellular protein, respectively. Both clones were grown in RPMI 1640 medium, supplemented with 10% (v/v) heat-inactivated fetal calf serum, 2 mM l-glutamine, and 0.5 mg/ml G418 (Invitrogen). Cells were cultured at 37 °C in a 5% CO₂, 95% air atmosphere. Cell culture plasticware was from Techno Plastic Products (Trasadingen, Switzerland). All other cell culture reagents were from Invitrogen.

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*K*, for **Inhibition of γGT by GSAO**—γGT activity was measured using γ-glutamyl-p-nitroanilide as substrate and glycylglycine as transpeptidation acceptor (11). γGT, γ-glutamyl-p-nitroanilide, glycyl-glycine, and GSAO in 15 mm Tris, pH 9.0, buffer were incubated at 25 °C in Linbro/Titertek E.L.A. microtitration plate wells. The reactions were started by the addition of γGT to a final volume of 200 μl. Reactant concentrations are found in the legend for Fig. 2. The formation of p-nitroaniline as a function of time was monitored continuously by measuring the absorbance at 405 nm using a Molecular Devices M2 Microplate Reader (Palo Alto, CA). The extinction coefficient used for p-nitroanilide was 9920 M⁻¹ cm⁻¹. The apparent *Kₘ* for inhibition of γGT by GSAO was estimated from the effect of fixed concentrations of GSAO on the initial velocity of hydrolysis of γ-glutamyl-p-nitroanilide by γGT. The results were consistent with simple competitive inhibition. The data points were globally fit by non-linear least squares regression using GraphPad (San Diego, CA) software.

**Production of GSAO Metabolites**—GSAO was produced as described previously to a purity >94% by HPLC (1). A 50 mM solution of GSAO was made by dissolving solid in 15 mM Tris, pH 7.4, buffer containing 40 mM glycyl-glycine. GCAO was produced by cleaving the γ-glutamyl group from GSAO with porcine kidney γ-glutamyl transpeptidase type IV (Sigma). A 10 mM solution of GSAO was incubated with 0.55 units/ml γGT in 15 mM Tris, pH 7.4, buffer containing 40 mM glycyl-glycine for 1 h at 30 °C. The γGT was removed from the reaction by filtration using a YM3 Microcon membrane (Millipore, Billerica, MA).

4-(N-(S-Cysteinylacetyl)amino) phenylarsonous acid (CAO) was produced by cleaving the glycine amino acid from GCAO with porcine kidney aminopeptidase N (Type IV-S, Sigma). The GCAO filtrate was incubated with 2 units/ml aminopeptidase N for 1 h at 37 °C. The aminopeptidase N was removed from the reaction by filtration using a YM3 Microcon membrane.

The concentrations of the metabolites were measured by titrating with dimercaptopropanol and calculating the remaining free thiols with 5,5'-dithiobis(2-nitrobenzoic acid) (1). The titrated solutions were sterile-filtered and stored at 4 °C in the dark until use. There was no significant loss in the active concentration of stock solutions of the arsenicals for at least a week when stored under these conditions.

**HPLC and Electrospray Mass Ionization-Mass Spectrometry Analysis of the Organoaarsenicals**—GSAO and metabolites were characterized by HPLC (1200 Series; Agilent Technologies, Santa Clara, CA). Samples were resolved on a Zorbax Eclipse XDB-C18 column (4.6 × 150 mm, 5 μm; Agilent Technologies) using a mobile phase of acetonitrile-water (25:75 v/v), flow rate of 0.5 ml/min, and detection by absorbance at 256 nm. The purity of GSAO, GCAO, and CAO by peak area was 94 ± 1, 69 ± 3, and 84 ± 4%, respectively.

Masses of the compounds were determined using electrospray mass ionization-mass spectrometry. Spectra were acquired using an API QStar Pulsar i hybrid tandem mass spectrometer (Applied Biosystems, Foster City, CA). Samples (~1 pmol) were loaded into nanospray needles (Proxeon), and the tip was positioned ~10 mm from the orifice. Nitrogen was used as curtain gas, and a potential of 900 V was applied to the needle. A time-of-flight mass spectrometry scan was acquired (m/z 200–2000, 1 s) and accumulated for ~1 min into a single file. The mass spectrum of GCAO gave molecular ions at 420.0 m/z [GCAO+H⁺]⁺, 402.0 m/z [GCAO+H₂O]⁺, and 383.9 m/z [GCAO+H₂O⁺]⁺, confirming the cleavage of the γ-glutamyl group from GSAO. The mass spectrum of CAO gave molecular ions at 362.9 m/z [CAO+H⁺]⁺, 384.9 m/z [CAO+Na⁺]⁺, and 344.9 m/z [CAO+H₂O⁺]⁺, confirming the cleavage of the glycine from GCAO.

**Accumulation of GSAO and Metabolites in BAE Cells**—BAE cells were seeded in Petri dishes (1.6 × 10⁶ cells) or 6-well plates (7.5 × 10⁵ cells) and allowed to attach overnight. The medium was replaced, and the cells were incubated for 30 min in the absence or presence of transport inhibitors. The cells were then incubated with GSAO, GCAO, or CAO, washed twice with ice-cold phosphate-buffered saline, and lysed with 1 ml of 70% w/w nitric acid. Lysates were diluted 30-fold and analyzed for
arsenic atoms using an Elan 6100 Inductively Coupled Plasma Spectrometer (PerkinElmer Sciex Instruments).

Mitochondrial Swelling Assay—Mitochondria were isolated from the livers of ~250-g male Wistar rats using differential centrifugation as described previously (1, 3). The final mitochondrial pellet was resuspended in 3 mM HEPES-KOH, pH 7.0, buffer containing 213 mM mannitol, 71 mM sucrose, and 10 mM sodium succinate at a concentration of 30 mg of protein/ml. Mitochondrial permeability transition induction was assessed spectrophotometrically by suspending the liver mitochondria at 0.5 mg of protein/ml at 25 °C in 3 mM HEPES-KOH, pH 7.0, buffer containing 75 mM mannitol, 250 mM sucrose, 10 mM sodium succinate, and 2 mM rotenone. Swelling was measured by monitoring the associated decrease in light scattering at 520 nm using a SpectraMax Plus microplate reader (Molecular Devices).

Statistical Analyses—Results are presented as means ± S.E. All tests of statistical significance were two-sided, and p values <0.05 were considered statistically significant.

RESULTS

Inhibition of Cell Surface γGT Blunts Accumulation of GSAO in Endothelial Cells and Antiproliferative Activity—Both the γGT substrate, reduced glutathione, and the γGT inhibitor, acivicin, significantly reduced accumulation of GSAO in endothelial cells (Fig. 1, B and C). Acivicin also blunted the antiproliferative activity of GSAO (Fig. 1D). These findings implied that the effect of GSAO on endothelial cells was modified by cell surface γGT.

To test this conclusion, the effect of varying cell surface expression of γGT on the antiproliferative activity of GSAO was assessed. Previously, well characterized c21 melanoma cell
clones expressing low (0.34 milliunits/mg of cell protein) or high (91 milliunits/mg of cell protein) levels of γGT activity were employed (10).

Expression Level of γGT Positively Correlates with Sensitivity of Model Cells to GSAO—Melanoma c21/basal and c21/γGT clones were tested for sensitivity to GSAO in 24- or 72-h assays. The 24-h GSAO IC_{50} (concentration of compound that inhibits proliferation by 50%) for proliferation arrest was ~60 and ~1250 μM for high and low γGT cells, respectively (Fig. 2A). These IC_{50} values fell to ~20 and ~115 μM, respectively, in 72-h assays (Fig. 2B). The antiproliferative effects of GSAO were negated in both high and low expressing cells with the potent γGT inhibitor, ABBA (Fig. 2C). Treatment with ABBA alone at the concentration used had no effect on cell proliferation or viability (data not shown).

These findings indicate that the susceptibility of cells to proliferation arrest by GSAO is markedly dependent on expression level of γGT. The time dependence of the antiproliferative effect suggests that GSAO is being cleaved by γGT and that the product(s) is then inhibiting cell proliferation. To test whether GSAO is a substrate for γGT, the apparent K_{i} for binding of GSAO to γGT was measured.

K_{i} for Binding of γGT to GSAO—Inhibition of γGT by GSAO was measured from the effect of fixed concentrations of GSAO on the initial velocity of hydrolysis of γ-glutamyl-p-nitroanilide by γGT. The apparent K_{i} for competitive inhibition of γGT by GSAO was estimated to be 1.6 ± 0.2 mm, which is similar to the K_{m} for hydrolysis of the chromogenic substrate, γ-glutamyl-p-nitroanilide (2.4 ± 0.4 mm) (Fig. 2D). This finding implies that the aminophenylarsanous acid moiety of GSAO does not demonstrably affect access of γGT to the glutathione pendant.

The OATP Family Is Involved in Endothelial Cell Uptake of the Product of GSAO Cleavage by γGT, GCAO—GCAO was produced by cleaving the γ-glutamyl group from GSAO with porcine kidney γ-glutamyl transpeptidase. The enzyme was removed from the reactions by size-exclusion filtration, and GCAO was analyzed by HPLC. GSAO and GCAO have retention times of 3.03 and 3.38 min, respectively (Fig. 3A). The organic anion-transporting polypeptide (OATP) family of transporters function independently of ATP and sodium gradients and were originally characterized as uptake transporters...
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FIGURE 5. The GCAO metabolite, CAO, accumulates in the endothelial cells at a similar rate and has comparable antiproliferative activity to GCAO. A, cleavage of GCAO by dipeptidase to produce CAO. B, C18 reverse phase HPLC analysis of 5 nmol of GCAO and CAO. C, CAO and GCAO accumulate in the cytosol at a similar rate. BAE cells were incubated with 50 μM GCAO or CAO for up to 4 h, and cystolic arsenic was measured by inductively coupled plasma spectrometry. The rates of accumulation are GCAO and CAO are 0.23 and 0.20 nmol of arsenic atoms/10^6 cells, respectively. Data points are the mean ± S.D. of three determinations, and the solid line is the linear least squares fit of the data. The results are representative of 2 experiments. D, GCAO and CAO IC_{50} values for proliferation arrest of endothelial cells. BAE cells were incubated with 0.8–100 μM GCAO or CAO for 24, 48, or 72 h. Cell viability was determined using MITT, and results were expressed as the percentage of untreated control. Values are mean ± S.D. of triplicate determinations. Results are representative of 3–5 experiments. ***, p < 0.001.

(12). OATP family members have been implicated in transport of glutathione-S conjugates (13), so they were candidate transporters for GCAO. 4,4'-Diisothiocyanostilbene-2,2'-disulfonic acid (DIDS) is an inhibitor of the plasma membrane OATP family of transporters. Accumulation of GCAO in endothelial cells (data not shown) and antiproliferative activity were reduced by DIDS (Fig. 3D), which implies that this family of transporters are involved in GCAO translocation across the plasma membrane.

GCAO Accumulates More Rapidly in Endothelial Cells and Has Greater Antiproliferative Activity than GSAO—GCAO accumulated in BAE cells at a 8.7-fold faster rate than GSAO (Fig. 3B). The rate of accumulation of GCAO was 3.8 ± 0.1 pmol of arsenic atoms/10^6 cells/min, when compared with 0.4 ± 0.1 pmol of arsenic atoms/10^6 cells/min for GSAO. The faster rate of accumulation of GCAO corresponded to a ~5-fold increased antiproliferative activity. The IC_{50} for GSAO and GCAO in a 24-h BAE cell proliferation assay was 97 ± 5 and 18 ± 3 μM, respectively (Fig. 3C). It is apparent from the results that the IC_{50} for GSAO markedly decreases with time of incubation and much less so for GCAO. For example, the 72-h GSAO IC_{50} is similar to the 24-h IC_{50} for GCAO. These results are consistent with a mechanism whereby GSAO is cleaved at the cell surface by γGT and the resultant GCAO enters the cell and triggers proliferation arrest.

GCAO Is Secreted from Endothelial Cells by MRP1—Cellular accumulation of GCAO is a balance between the rate of uptake and the rate of export from the cell. GSAO accumulation in cells is controlled by the rate of export by MRP1 and MRP2 (2). To test whether GCAO is also exported by MRPI, the effect of the MRP1 inhibitor 4H10 on accumulation in endothelial cells was measured. Cellular accumulation of GCAO was increased 3-fold when MRPI was inhibited (Fig. 4A), which correlated with the more potent antiproliferative effect (Fig. 4B). The inhibitor alone had no effect on BAE cell proliferation (data not shown).

GCAO Triggers the Mitochondrial Permeability Transition More Rapidly than GSAO—GSAO inactivates the mitochondrial inner membrane transporter ANT, which leads to proliferation arrest and cell death (1). GCAO also induces the mitochondrial permeability transition (Fig. 4C). Comparison of the
time for 25% maximal swelling as a function GSAO or GCAO concentration indicates that GCAO is approximately twice as efficacious as GSAO at triggering the permeability transition (Fig. 6C).

**DISCUSSION**

γGT is present on the outer surface of the plasma membrane (14) of virtually all cells. The enzyme catalyzes hydrolysis of the bond linking the glutamate and cysteine residues of extracellular glutathione and glutathione-S conjugates (15). GSAO is effectively a glutathione-S conjugate of aminophenylarsanous acid, so it is not surprising that it is a substrate for γGT. This has been demonstrated in several ways.

First, GSAO is an efficient substrate for isolated γGT. Second, endothelial cell accumulation and antiproliferative activity of GSAO was blunted by extracellular glutathione and an active site inhibitor of γGT. The glutathione likely acted as a competitive substrate inhibitor of γGT in this experiment. Third, the level of cell surface γGT correlated strongly with the sensitivity of model cells to GSAO.

There was a marked time dependence of the antiproliferative effect of GSAO on endothelial cells. The longer the incubation of GSAO with γGT-expressing cells, the lower the IC50 for proliferation arrest. This time dependence is consistent with a mechanism where GSAO is cleaved by γGT and the product of this reaction is inhibiting cell proliferation. This mechanism was tested by making the product of γGT cleavage, GCAO, and examining how endothelial cells respond to this compound. As anticipated, GCAO accumulated much more rapidly in endothelial cells than GSAO and had greater antiproliferative activity.

The mechanism of GCAO import and export from endothelial cells was examined. Import of GCAO into endothelial cells (data not shown) and antiproliferative activity was reduced by an inhibitor of the OATP family (13). Some transporters are proton- or sodium-dependent, including the proton-coupled oligopeptide transporter family (16) and the sodium-dependent glutathione transport mechanism and sulfate dicarboxylate transporter (17). GCAO accumulation in endothelial cells was not activated at low pH and was only marginally sodium-dependent (data not shown), however, implying that these transporters are not involved in GCAO uptake. Export of GCAO, as for GSAO (2), is via MRP1 and likely also MRP2. Glutathione-S conjugates and their metabolites are known substrates for these MRPs (2, 18).

The glycine-cysteine peptide bond of GCAO is readily cleaved by dipeptidases to produce CAO. A number of dipeptidases have been identified as potential cleavage sites, including dipeptidyl peptidase IV (19) and dipeptidyl peptidase IX (20). These enzymes are known to cleave the glycine-cysteine bond and release CAO from GCAO (21). Therefore, it is likely that dipeptidases play a role in the metabolism of GCAO in endothelial cells.

**FIGURE 7. Metabolism of GSAO by endothelial cells.** The γ-glutamyl residue of GSAO is cleaved at the cell surface by γ-GT to produce GCAO, which is then transported across the plasma membrane by an OATP. GCAO may be further processed to CAO before it reacts with ANT of the inner mitochondrial membrane.
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tidases are found on the plasma membrane (19) and in the cytosol (20). It is likely that GCAO is cleaved on or in the cell by one or more dipeptidases. CAO accumulated in endothelial cells at the same rate as GCAO and had comparable antiproliferative activity. Like GSAO and GCAO, CAO was also a substrate for MRP1 in endothelial cells. These findings suggest that further processing of GCAO to CAO has little bearing on how endothelial cells respond to the compound.

A number of trivalent arsenicals, including sodium arsenite (21), phenylarsonous acid (4), GSAO (1), and ortho-GSAO (3), have been shown to react with two cysteine thiols on the matrix face of mitochondrial ANT, forming stable cyclic dithioarsinite complexes in which both sulfur atoms are bound to arsonic. This interaction triggers opening of the mitochondrial transition pore, which leads to proliferation arrest and apoptotic cell death. CAO was about two times more efficacious than GCAO and GCAO was about two times more effective than GSAO at triggering the mitochondrial permeability transition in isolated mitochondria. GSAO and its metabolites, therefore, have similar effects on mitochondrial integrity.

There is precedent for an important role for γGT in the mechanism of action of glutathione-S conjugates other than GSAO. γGT-dependent metabolism of glutathione-S-hydroxy-2,3-trans-nonenal is associated with a considerable increase of cytotoxicity due to the release of cysteinyl-glycine-S-hydroxy-2,3-trans-nonenal (15). Also, γGT processing of mercuric glutathione conjugate to mercuric cysteinyl-glycine results in increased luminal uptake of the product in the proximal tubules of the kidney (19).

The pro-drug mechanism of action of GSAO described here implies that GSAO should be more effective against γGT-positive tumors. Metabolism of GSAO by tumor cell and/or tumor endothelium γGT would produce high local concentrations of GCAO that will then block tumor angiogenesis and tumor growth. Notably, tumors of the breast, prostate, colon, liver, and ganglion cells of the sympathetic nervous system (16) are known to have higher levels of γGT activity than soft tissue tumors (17). Tumors tend to have higher levels of GSAO administration resulted in damage to the distal tubules of the kidney in mice and rats. The damage resolved upon cessation of treatment. Interestingly, high levels of γGT and dipeptidase (dehydropeptidase 1) have been reported in kidney proximal tubules (19, 22). Moreover, the GCAO and CAO transporter OATP is expressed specifically on the apical side of distal nephrons (23). The kidney toxicity observed at maximum tolerated doses of GSAO in rodents, therefore, may be a consequence of high local processing of GSAO by γGT in the proximal tubules and transport of GCAO into the distal tubule cells, where it triggers mitochondrial-mediated apoptosis.

Taken together, our findings indicate that γGT processing of GSAO at the cell surface is the rate-limiting step in its antianгиogenic activity (Fig. 7). The reaction product GCAO is then transported into the cell by OATP, where it may be further processed to CAO before it reacts with its mitochondrial target. This information will aid in the design of better antimitochondrial cancer drugs.

Acknowledgments—We thank Russell Pickford for helpful discussion and the mass spectrometry spectra analysis. We are grateful to Mark Raftery from the Bioanalytical Mass Spectrometry Facility and Rabeya Akter from the UNSW Analytical Centre for mass spectrometry and inductively coupled plasma spectrometry, respectively. We also thank Shane Thomas for assistance with HPLC.

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