Para to Ortho Repositioning of the Arsenical Moiety of the Angiogenesis Inhibitor 4-((N-(S-Glutathionylacetyl)Amino)Phenylarsenoxide Results in a Markedly Increased Cellular Accumulation and Antiproliferative Activity

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Abstract

The synthetic tripeptide arsenical 4-(N-(S-glutathionylacetyl) amino)p-phenylarsenoxide (p-GSAO) is an angiogenesis inhibitor that inactivates mitochondrial adenine nucleotide translocase (ANT) by cross-linking a pair of matrix-facing cysteine residues. This causes an increase in superoxide levels and proliferation arrest of endothelial cells followed by mitochondrial depolarization and apoptosis. p-GSAO induces proliferation arrest in endothelial cells and is a selective inhibitor of endothelial cells compared with tumor cells. An analogue of p-GSAO has been made in which the arsical moiety is at the othoro instead of the para position on the phenyl ring. o-GSAO, like p-GSAO, bound to ANT in a dithiol-dependent manner but was ~8-fold more efficient than p-GSAO at triggering the mitochondria permeability transition in isolated mitochondria. o-GSAO was an ~50-fold more potent inhibitor of endothelial and tumor cell proliferation than p-GSAO. The mechanism of this effect was a consequence of ~300-fold faster rate of accumulation of o-GSAO in the cells, which is due, at least in part, to impaired export by the multidrug resistance–associated protein 1. Administration of o-GSAO to tumor-bearing mice delayed tumor growth by inhibiting tumor angiogenesis but there were side effects not observed with p-GSAO administration. (Cancer Res 2005; 65(24): 11729-34)

Introduction

We recently described the organoarsenical 4-((N-(S-glutathionylacetyl)amino)p-phenylarsenoxide (p-GSAO), which inhibits proliferating, but not growth-quiescent, endothelial cells in vitro and angiogenesis in vivo (1, 2). p-GSAO inactivates the mitochondrial adenine nucleotide translocase (ANT) that exchanges matrix ATP for cytosolic ADP across the inner mitochondrial membrane and is the key component of the mitochondrial permeability transition pore (3–6). The trivalent arsenical moiety of GSAO cross-links the matrix facing Cys160 and Cys257 thiols of ANT (1) and effectively locks ANT into an open configuration. Inactivation of ANT by p-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 Ca2+ levels in proliferating cells. ANT is a Ca2+ receptor and p-GSAO binds with higher affinity to Ca2+-replete than to Ca2+-depleted ANT. The crystal structure of the calcium-free form of bovine ANT supports this mechanism (7). The cysteine pair that GSAO cross-links in the calcium-replete form are 18.2 Å apart in the calcium-free structure, which is at least twice the distance required for them to interact with trivalent arsenicals (8, 9).

p-GSAO is also a selective inhibitor of proliferating endothelial cells compared with tumor cells (1). The p-GSAO IC50 for proliferation arrest of endothelial cells is 3- to >32-fold lower than for different tumor cells. This selectivity is a consequence of the different rates of export of p-GSAO from the cells. p-GSAO is likely conjugated to one or two molecules of reduced glutathione and the complex is then eliminated from cells by the multidrug resistance–associated proteins (MRP) 1 or 2 (10). Tumor cells export p-GSAO much more efficiently than endothelial cells because they have higher MRPl or MPR2 activity and cellular glutathione levels (10).

These in vitro observations reflect the in vivo effects of p-GSAO. Systemic administration of p-GSAO to mice bearing s.c. tumors reduced the vascular density and growth rate of the tumors but had no measurable effect on the tumor cells themselves. There are no signs or symptoms of toxicity of p-GSAO in mice when administered at efficacious doses. A key aspect of p-GSAO is the glutathione moiety. This highly charged pendant safely delivers the arsenical to endothelial cell mitochondria. p-GSAO will be tested in a phase I/IIa clinical trial in cancer patients in 2006.

In an effort to better understand the mechanism of action of this organoarsenical and to refine its efficacy as an anticancer drug, we have made an analogue in which the position of the arsenical moiety of GSAO is at the ortho, not the para, position on the phenyl ring (Fig. 1). We thought that the ortho positioning of the arsenical might improve its dithiol reactivity. The electrophilic character of the arsenic atom is predicted to be enhanced through formation of a six-membered intramolecular hydrogen bond between the oxygen of the arsenic-bound hydroxyl and the hydrogen of the NH group. o-GSAO is a more potent inhibitor of endothelial cell proliferation than p-GSAO but administration to tumor-bearing mice resulted in side effects not seen with p-GSAO. These effects can be explained by increased accumulation of o-GSAO in endothelial cells.

Materials and Methods

Synthesis and purification of o-GSAO. o-Arsanic acid (4.70 g, 21.7 mmol) was dissolved in 1.85 mol/L KOH (25 mL), treated with Na2CO3 (6 g), and then diluted with H2O (25 mL). The solution was cooled in ice for 30 minutes, then added to a 250-mL separating funnel. A solution of bromoacetyl bromide (4 mL, 46.0 mmol) in CH2Cl2 (20 mL) was added cautiously to the separating funnel with frequent shaking and release of

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CO₂ (g). Addition took about 30 minutes. After CO₂ evolution had ceased, the CH₂Cl₂ was separated and the aqueous layer was acidified with concentrated H₂SO₄. A white precipitate resulted which was collected by filtration (6 g, 82% yield). The resulting 2-(2-bromoacetyl)amino)benzenearsonic acid (6 g, 17.8 mmol) was dissolved in HBr/methanol (1:1), NaI (80 mg) was added and the reaction was purged with N₂ and cooled to 0°C. SO₂ was bubbled through at ca. 4 bubbles/s and after 15 minutes, a white precipitate started to form. SO₂ was bubbled through for a further 2.5 hours as the reaction was warmed to room temperature. The reaction was purged with N₂ for 10 minutes and the solid was collected by filtration washing with H₂O (×3), ether (×2) to give 1.35 g of 2-(2-bromoacetyl)amino)benzenearsonic acid. A further 0.25 g was obtained from the initial filtrate. Total yield was 1.60 g (28%). 2-(2-Bromoacetylaminobenzenearsonic acid (288 mg, 0.894 mmol) was suspended in degassed H₂O (5 mL). NEt₃ (250 µL) was added, followed by glutathione (160 mg, 0.521 mmol). The white suspension was stirred vigorously under N₂ for 20 hours. The reaction was filtered through a glass-fritted filter and the filtrate was concentrated on a rotary evaporator. The residue was transferred to a plastic tube and lyophilized for 1.5 hours, resulting in a white precipitate.

The structure of o-GSAO was confirmed by liquid chromatography-mass spectrometry and by ¹H nuclear magnetic resonance. Purity was 97.7% by high-performance liquid chromatography. The molecular weight is 548.4.

p-GSAO and 4-((N-(S)-(6-((biotinoyl)amino)hexanoyl)amino)hexanoyl)glutathionylhexacacetil)aminophenylarsenoxide (p-GSAO-B) were prepared as described by Don et al. (1). The ortho derivative of p-GSAO-B was prepared by substituting o-GSAO for p-GSAO in the synthesis.

p-GSAO and p-GSAO-B were dissolved in deoxygenated PBS and concentrations were determined by titrating with dimercaptotripolysulfane and calculating the remaining free thiols with 5,5’-dithiobis(2-nitrobenzoic acid) (9). The conjugates 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 month when stored under these conditions.

Mitochondrial swelling assay. Mitochondria were isolated from the livers of 250-g male Wistar rats using differential centrifugation as previously described (1,11). The final mitochondrial pellet was resuspended in 3 mmol/L HEPES-KOH buffer (pH 7.0) containing 213 mmol/L mannitol, 71 mmol/L sucrose, and 10 mmol/L sodium succinate at a concentration of 30 mg protein/mL. Mitochondrial permeability transition induction was assessed spectrophotometrically by suspending the liver mitochondria at 0.5 mg protein/mL at 25°C in 3 mmol/L HEPES-KOH buffer (pH 7.0) containing 75 mmol/L mannitol, 250 mmol/L sucrose, 10 mmol/L sodium succinate, and 2 µmol/L rotenone (12). Swelling was measured by monitoring the associated decrease in light scattering at 520 nm using a SpectraMax Plus microplate reader (Molecular Devices, Sunnyvale, CA).

Binding of o-GSAO to adenine nucleotide translocase. Rat liver mitochondria were suspended at 2 mg protein/mL in 3 mmol/L HEPES-KOH buffer (pH 7.0) containing 213 mmol/L mannitol, 71 mmol/L sucrose, and 10 mmol/L sodium succinate and incubated with 10 µmol/L o-GSAO-B or p-GSAO-B in the absence or presence of 50 µmol/L 2,5-dimercaptopyrrolopanol (Fluka, Buchs, SG, Switzerland) for 1 hour at room temperature on a rotating wheel. The mitochondria were washed once with buffer, then sonicated in 0.3-mL ice-cold 25 mL/L Trits buffer (pH 7.4) containing 140 mmol/L NaCl, 2.7 mmol/L KCl, 0.5% Triton X-100, 0.05% Tween 20, 3 mol/L urea, 10 µmol/L leupeptin, 1 µmol/L aprotinin, 50 mg/mL 4-2-(aminoethyl)-benzene sulfonil fluoride, and 5 mL/L EDTA. Lysate was clarified by centrifugation at 18,000 × g for 10 minutes at 4°C and incubated with 30 µL of streptavidin-coated Dynabeads (Dynal, Oslo, Norway) for 2 hours at 4°C to isolate the biotin-labeled proteins. The beads were washed twice with sonication buffer and twice with 25 mL/L Trits buffer containing 140 mmol/L NaCl and 0.5% Triton X-100. Proteins bound specifically to GSAO arsenical moiety were eluted from the beads by incubation with 40 mL of 25 mL/L Trits buffer containing 50 mL/L DTT for 5 minutes (10). The eluted proteins were resolved on 8% to 16% gradient iGels (Gradipore, Sydney, Australia) under reducing conditions and transferred to polyvinylidene difluoride membrane. Proteins were detected by Western blot using a 1:500 dilution of goat anti-human AN polyclonal antibody (Santa Cruz Biotechnology, Santa Cruz, CA) and 1:2,000 dilution of rabbit anti-goat peroxidase-conjugated antibodies (DAKO).
Centre). Mice were held in groups of three to five at a 12-hour day/night cycle and were given animal chow and water *ad libidum*. A suspension of 2 x 10^6 BxPC-3 cells in 0.2 mL of PBS was injected s.c. in the proximal midline. Tumors were allowed to establish and grow to a size of 40 mm^3; after which they were randomized into four groups. Tumor volume was calculated using the relationship length ^2 * width for each animal. Tumor volume and animal weight were measured every 2 or 3 days.

**Immunohistochemistry.** Blood vessel density was estimated by immunostaining sectioned tumors for CD31 (rat anti-mouse CD31, BD PharMingen, North Ryde, Australia). Excised tumors were frozen and 4-μm sections were cut, mounted on Superfrost slides, fixed in cold acetone for 5 minutes, and incubated in 3% H2O2 in methanol for 15 minutes to block endogenous peroxidase. Tumor cell proliferation was estimated by immunostaining for proliferating cell nuclear antigen (PCNA; mouse anti-human PC10, DAKO). Excised tumors were fixed in formalin, embedded in paraffin, and 4-μm sections were cut and mounted on Superfrost slides. Sections were deparaffinized in xylene, rehydrated in PBS using an ethanol gradient, and microwaved for 5 minutes to increase antigen retrieval. Sections were incubated with anti-CD31 at 1:50 dilution and with anti-PCNA-positive cells in 100 tumor cells in three random fields in each tumor tissue. Magnification was ×400.

**Statistical analyses.** Results are presented as means ± SE. All variables were examined for normality and homogeneity of variance. Normality of the distributions was assessed using the Shapiro-Wilk test. For the tumor growth studies, multiple comparisons were made between the various treatment groups to determine whether they differed across treatment groups using the multivariate linear model (15). Data of blood vessel density and tumor cell proliferative index were analyzed using one-way ANOVA. Post hoc tests, parametric or nonparametric as appropriate, were used to compare mean values. Tumor doubling time was analyzed with the log-rank test (15). All analyses were done using the statistical package SPSS (version 13). All tests of statistical significance were two sided and *P < 0.05* was considered statistically significant.

**Results**

- **o-GSAO** is more efficient than *p*-GSAO at inducing the mitochondrial permeability transition. The effects of *ortho* repositioning of the arsenical moiety in GSAO on mitochondrial permeability transition pore induction were determined. *o*-GSAO, like *p*-GSAO, triggered swelling of isolated rat liver mitochondria in a time- and concentration-dependent manner (Fig. 2A and B). The time for half-maximal swelling of isolated mitochondria was three to five times faster for a given concentration of *o*-GSAO compared with *p*-GSAO (Fig. 2C). Indeed, *o*-GSAO was almost as efficient as the lipophilic phenylarsenoxide at inducing the permeability transition (Fig. 2D).

- **Interaction of *o*-GSAO with adenine nucleotide translocase.** Mitochondrial permeability transition pore induction is controlled by the binding of Ca^{2+}, cyclophilin D, and adenine nucleotides to ANT (for review, see ref. 3). An increase in matrix Ca^{2+} concentration is the main trigger for opening of the mitochondrial permeability transition pore. Chelation of Ca^{2+} with EGTA or an excess of divalent metal ions such as Mg^{2+} blocks pore opening. Both EGTA and Mg^{2+} blocked *o*-GSAO–dependent swelling (Fig. 3A). Binding of cyclophilin D to ANT is necessary for pore opening at submillimolar Ca^{2+} concentrations and cyclophilin A blocks pore opening by binding to cyclophilin D and displacing it from ANT. ADP inhibits pore opening by binding to ANT and increasing the concentration of Ca^{2+} required to trigger pore opening (3, 4). Both cyclophilin A and ADP blocked the effect of *o*-GSAO on pore opening (Fig. 3A).

These observations are analogous to the findings with *p*-GSAO (1) and implied that ANT is also the key mitochondrial target of *o*-GSAO. This conclusion was tested by measuring the binding...
of a biotin-tagged o-GSAO to ANT. Isolated mitochondria were incubated with biotin-tagged p-GSAO or o-GSAO in the absence or presence of a 5-fold molar excess of 2,3-dimercaptopropanol and the labeled proteins were collected on streptavidin-coated beads. Both p-GSAO and o-GSAO bound to ANT in a dithiol-dependent manner (Fig. 3B).

Ca²⁺ dependence of o-GSAO–mediated transition pore opening. The rate of o-GSAO–mediated mitochondrial permeability transition pore induction was a function of Ca²⁺ concentration in the physiologic range (Fig. 4A and B). For example, the time for half-maximal swelling in the presence of 2 μmol/L o-GSAO decreased from 53 minutes at 1 μmol/L Ca²⁺ to 15 minutes at 10 μmol/L Ca²⁺ (Fig. 4B). Essentially the same Ca²⁺ dependence was observed for p-GSAO and o-GSAO although o-GSAO was an ~8-fold more potent activator of the pore than p-GSAO in the 5 to 10 μmol/L range. For example, the time for half-maximal swelling in the presence of 10 μmol/L p-GSAO or o-GSAO and 5 μmol/L Ca²⁺ was 8 and 59 minutes, respectively (Fig. 4C).

Antiproliferative activity of o-GSAO. Reported IC₅₀ values for proliferation arrest and loss of viability of BAE cells induced by p-GSAO are 10 and 75 μmol/L, respectively (1). This finding indicates that GSAO arrests proliferation of BAE cells at ~7-fold lower concentrations than are required to induce apoptosis.

IC₅₀ values for proliferation arrest and loss of viability of BAE cells by o-GSAO were 0.2 ± 0.1 and >3 μmol/L, respectively (Fig. 5A and C). o-GSAO also had no effect on viability of growth-quiescent BAE cells at concentrations five times the IC₅₀ for proliferation arrest (Fig. 5B). o-GSAO was ~50 times more effective than p-GSAO at arresting the proliferation of BAE cells (IC₅₀ of 0.2 ± 0.1 μmol/L for o-GSAO and 9 ± 1 μmol/L for p-GSAO; Fig. 5C). o-GSAO, like p-GSAO, was a selective inhibitor of BAE compared with BxPC-3 cell proliferation. The IC₅₀ for proliferation arrest of BxPC-3 cells was 13 ± 2 and >300 μmol/L for o-GSAO and p-GSAO, respectively (Fig. 5D).

Uptake of o-GSAO by endothelial cells. A possible mechanism for the increased antiproliferative activity o-GSAO compared with p-GSAO was increased accumulation in cells. This theory was tested by comparing the uptake of p-GSAO and o-GSAO in BAE cells by measuring cellular accumulation of GSAO-derived arsenic. o-GSAO accumulated in BAE cells at an ~300-fold faster rate than p-GSAO (Fig. 6A). The initial rates of accumulation of p-GSAO and o-GSAO were 1.08 and 310 pmol/10⁶ cells/min, respectively. The decrease in o-GSAO accumulation after 60 minutes was due to the cytotoxicity of this compound. Approximately 50% of the cells had detached at 60 minutes.

MRP1, in combination with cellular glutathione, mediates export of p-GSAO from BAE cells (10). We hypothesized that the increased accumulation of o-GSAO in BAE cells was due to decreased efflux via MRP1. This theory was tested by using the MRP inhibitor MK-571. Treatment of BAE cells with MK-571 caused a dose-dependent decrease in both the p-GSAO and o-GSAO IC₅₀ (Fig. 6B). The p-GSAO IC₅₀ decreased by 12-fold, 12 ± 1 to 1.0 ± 0.2 μmol/L, whereas the o-GSAO IC₅₀ decreased by only 4-fold, 0.18 ± 0.04 to 0.04 ± 0.01 μmol/L (Fig. 6C). The highest concentration of MK-571
employed, 20 μmol/L, had no effect BAE cell proliferation. This result indicates that both p-GSAO and o-GSAO are exported from BAE cells by MRP1 but that o-GSAO is transported less efficiently than p-GSAO.

Antitumor activity of o-GSAO. There are no apparent adverse affects of s.c. administration of p-GSAO to either severe combined immunodeficient or C57BL/6J mice (1). To assess the toxicity of o-GSAO, 10 mg/kg/d was administered s.c. to BALB/c mice. There were occasional short-term behavioral side effects (ruffled fur, hyperactivity, and clonic movements of the hind limbs) following administration of o-GSAO and also thickening of the skin and occasional necrosis with repeated injections in the same area.

The growth of established human primary tumors in immunocompromised mice was suppressed by administration of o-GSAO. Female, 7- to 9-week-old BALB/c nude mice bearing ~40-mm³ s.c. BxPC-3 tumors were administered vehicle (PBS) or 1 or 10 mg/kg/d p-GSAO (Fig. 7A) or o-GSAO (Fig. 7B) by s.c. injection at a site remote from the tumor. Treatment with 1 or 10 mg/kg/d o-GSAO increased the tumor doubling time from 6.8 ± 0.6 days (vehicle) to 9.1 ± 1.7 and 15.7 ± 4.2 days (P < 0.05), respectively. Treatment with 10 mg/kg/d p-GSAO increased the tumor doubling time from 6.8 ± 0.6 days (vehicle) to 10.7 ± 2.1 days (P < 0.05). The average body weight of the 1 mg/kg/d o-GSAO treatment group over the course of the experiment was not significantly different from vehicle control and there were no macroscopic differences or morphologic changes apparent in the brain, heart, lungs, liver, kidneys, and spleen of the treated mice (data not shown). However, in 9 of 11 mice, there was a mild mononuclear inflammation in the skin at the site of injection. Treatment was discontinued in the 10 mg/kg/d o-GSAO treatment group at day 12 due to a decline in body weight and signs of toxicity at the injection site ranging from skin thickening to necrosis (data not shown).

The tumors were excised at the conclusion of treatment (19 days) and examined histologically for blood vessel density and tumor cell proliferation. There was a significant reduction in blood vessel density in the 1 mg/kg/d o-GSAO tumors (P < 0.001; Fig. 7C) but the proliferative index of the tumor cells was the same as control (Fig. 7D).

Discussion

p-GSAO is an effective antiangiogenic agent because of two key features: its targeting of proliferating and not growth-quiescent endothelial cells and its selectivity for endothelial compared with other proliferating cells. The mechanistic bases for these properties are reasonably well understood. The specificity of p-GSAO for proliferating endothelial cells is due, at least in part, to higher mitochondrial Ca²⁺ levels in proliferating cells. The target of p-GSAO, ANT, is a Ca²⁺ receptor and p-GSAO binds with higher affinity to ANT when the translocase has bound Ca²⁺ (1). The selectivity of p-GSAO for endothelial cells is due to inefficient export of GSAO from these cells, which is a consequence of low MRP1 activity and cellular glutathione levels (10). Tumor cells, for
instance, are more resistant to GSAO because they have higher MRP1 or MRP2 activity and cellular glutathione, which combine to export GSAO. The consequences of repositioning of the arsenical moiety on the phenyl ring to the para to ortho position for antiangiogenic activity have been characterized in this study. o-GSAO, like p-GSAO, bound to ANT in a dithiol-dependent manner and triggered the formation of the mitochondria permeability transition pore although the half-time for induction of the pore in isolated mitochondria was three to five times faster for a given concentration of o-GSAO compared with p-GSAO. The rate of o-GSAO–mediated pore induction was also a function of Ca\(^{2+}\) concentration in the physiologic range. A similar Ca\(^{2+}\) dependence was observed for p-GSAO and o-GSAO although o-GSAO was an ~8-fold more potent activator of the pore than p-GSAO in the 5 to 10 \(\mu\text{mol/L}\) Ca\(^{2+}\) range. Notably, o-GSAO was an ~50-fold and 25-fold more potent inhibitor of endothelial cell and pancreatic carcinoma tumor cell proliferation, respectively, than p-GSAO. o-GSAO, like p-GSAO, was a selective inhibitor of endothelial cell proliferation compared with the pancreatic carcinoma tumor cell line, with an IC\(_{50}\) for proliferation arrest 67-fold higher for the tumor cell line.

The mechanism of the increased effect of o-GSAO relative to p-GSAO on cell proliferation was likely due to the ~300-fold faster rate of accumulation of o-GSAO in the cells. This faster accumulation can be explained to some extent by the reduced efficiency of export of o-GSAO by MRP1. Our findings suggest that the export of o-GSAO from endothelial cells via MRP1 was ~3-fold less efficient than export of p-GSAO by this transporter. Human MRP1 transports As(III) as a complex with three molecules of reduced glutathione (16) and it is likely that the arsenical moiety of p-GSAO similarly reacts with one or two molecules of glutathione before it is removed by MRP1 (10). There are two possible reasons why o-GSAO is insufficiently exported by MRP1: the arsenical moiety may be poorly glutathionylated due to its position on the phenyl ring or the o-GSAO-(GSH)\(_n\) complex is a poorer substrate for MRP1 than p-GSAO-(GSH)\(_n\) \((n\) represents the number of glutathione molecules, which is likely to be 1 or 2). It is also possible that the increased accumulation of o-GSAO compared with p-GSAO in endothelial cells was due, in part, to a faster rate of entry. The mechanism of entry of these organoarsenicals into cells is not known.

The enhanced rate of accumulation of o-GSAO in endothelial cells was expected to influence its antiangiogenic activity in vivo and perhaps result in side effects not observed with p-GSAO. This was found to be the case. Treatment of immunocompromised mice bearing s.c. human pancreatic carcinoma tumors with 10 mg/kg/d s.c. injections of o-GSAO inhibited tumor growth by ~70% after 14 days of treatment but there were signs and symptoms of toxicity of the compound. There were occasional short-term behavioral side effects following administration of o-GSAO: skin thickening to necrosis at the site of injection and decline in body weight after 12 days of treatment. It is possible that o-GSAO disrupted angiogenesis associated with wound healing at the injection site.

Despite an IC\(_{50}\) of 13 \(\mu\text{mol/L}\) for proliferation arrest of the pancreatic tumor cell line in vitro, there was no evidence for an antitumor cell effect of o-GSAO in vivo. A decrease in vascularity of the treated tumors was observed but there was no change in the proliferative index of the tumor cells or signs of tumor necrosis. This analysis indicated that o-GSAO, like p-GSAO, was acting as an angiogenesis inhibitor and had little or no antitumor cell activity. This is likely a consequence of the pharmacokinetics of the compounds. Both compounds are soluble in neutral pH water at concentrations >100 mg/mL. This high water solubility seems to restrict their distribution in vivo largely to the intravascular compartment.

In summary, o-GSAO is more efficacious than p-GSAO in vitro and in vivo but has side effects that are not apparent with p-GSAO administration. The main consequence we observed of repositioning of the arsenical moiety from the para to ortho position on the phenyl ring is markedly increased accumulation in cells, which relates to decreased export by MRP1. The slower rate of accumulation of p-GSAO in cells may account for its lack of toxicity in vivo at efficacious doses.

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