GLUTATHIONE AND MERCAPTURIC ACID CONJUGATES OF SULOFENUR AND THEIR ACTIVITY AGAINST A HUMAN COLON CANCER CELL LINE

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ABSTRACT:
Sulofenur is one of the diarylsulfonylureas developed as an anticancer agent. Sulofenur possesses a broad spectrum of activity in several solid tumor models and has undergone extensive clinical trials based on its impressive preclinical activity. However, the clinical response of sulofenur has been disappointing because of the side effect of anemia. Furthermore, the anticancer mechanism of sulofenur and its diarylsulfonylurea analogs still remains unknown. Elucidation of the metabolic fates of sulofenur may help to delineate the mechanism and provide information to guide the structural modification for more potent anticancer agents with less side effects. We have identified a glutathione conjugate and a mercapturic acid conjugate from sulofenur-dosed rats with the aid of liquid chromatography/mass spectrometry. The fraction of the dose of sulofenur as the glutathione conjugate in the dosed-rat bile over 5 h was 0.12 ± 0.03%, and the mercapturic acid conjugate in urine over 24 h was 1.4 ± 0.7%. Protein binding of the glutathione conjugate and mercapturic acid conjugate was determined to be 20 ± 3 and 84 ± 2%, respectively, as opposed to >99% of sulofenur. The high protein binding of sulofenur requires a higher than in vitro dose, which is believed to cause the side effect of anemia. The significance of this metabolic pathway is that both conjugates were found to be glutathione reductase inhibitors and to possess anticancer activity comparable to sulofenur against human colon adenocarcinoma GC2/c1 cells, a sulofenur-sensitive cell line. These conjugates may serve as new leads for the development of novel anticancer agents.

Sulofenur (Fig. 1) is one of the diarylsulfonylureas developed as a potent anticancer agent (Howbert et al., 1990; Mohamadi et al., 1992). The compound exhibits a broad spectrum of activity in rodent solid tumors and human tumor xenografts (Mohamadi et al., 1992). However, clinical trials of sulofenur have yielded unsatisfactory results because of the high protein binding and dosing being limited by the appearance of anemia due to methemoglobinemia, a side effect likely associated with its aniline-related metabolites (Houghton and Houghton, 1996). Furthermore, despite extensive research efforts, the underlying mechanism(s) of the anticancer activity of sulofenur, as well as other diarylsulfonylureas, remain unsolved (Houghton and Houghton, 1996). Elucidation of the metabolic fates of sulofenur may help our understanding of the anticancer mechanism(s) of the drug and guide the structural modification to reduce side effects.

The primary metabolic pathways of sulofenur in humans and other species are oxidation of the saturated carbons in the indane ring (Ehlhardt, 1991, 1992). Other reported metabolites include p-chloroaniline and its metabolites, 2-amino-5-chlorophenyl sulfate and p-chloro-oxanilic acid (Ehlhardt, 1991). No anticancer activity has been reported with these metabolites.

We have noticed, based on our earlier work (Guan et al., 1994, 1999) and related work from our colleagues (Davis et al., 1993; Slatter et al., 1993; Jochheim and Baillie, 1994), that compounds with a urea structural feature [–(N or R′)-C(O)-NH-R] are likely to undergo metabolic conversion to generate a corresponding glutathione conjugate, GS-(C-O)-NH-R (Fig. 2). Although this is a minor metabolic pathway, the glutathione conjugate may play a role in the biological or toxicological effects of the parent drug (Davis et al., 1993; Slatter et al., 1993; Guan et al., 1994, 1999; Jochheim and Baillie, 1994). This is because of the fact that these glutathione conjugates are all irreversible glutathione reductase (GR) inhibitors (Davis et al., 1993; Guan et al., 1994, 1999; Jochheim and Baillie, 1994). Although the mechanism of glutathione conjugation from the substituted ureas is not fully understood, it has been proposed that the formation is likely to go through an isocyanate intermediate (Davis et al., 1993; Slatter et al., 1993; Guan et al., 1994, 1999; Jochheim and Baillie, 1994). Sulofenur also has a urea structural feature of [–(N or R′)-C(O)-NH-R, R′ = p-chlorophenyl]. Therefore, it is likely that sulofenur is metabolized to the corresponding glutathione conjugate [GS-(C-O)-NH-R, R′ = p-chlorophenyl]. Based on earlier reports (Davis et al., 1993; Guan et al., 1994, 1999; Jochheim and Baillie, 1994), it is also

1 Abbreviations used are: GR, glutathione reductase; GSH, glutathione; LC/MS, liquid chromatography/mass spectrometry; BSA, bovine serum albumin; BCNU, N, N-Bis(2-chloroethyl)-N-nitrosourea; HPLC, high-performance liquid chromatography.
reasonable to predict that this glutathione conjugate could be an irreversible GR inhibitor. GR plays a critical role in maintaining the intracellular reduced form of glutathione (GSH) levels (Deneke and Fanburg, 1989; Meister, 1991). Inhibition of GR has been demonstrated to deplete intracellular GSH (Kassahun et al., 1994). In view of the important role of GSH in maintaining cell integrity (Meister and Anderson, 1983), inhibition of GR may produce cell growth inhibition. As a matter of fact, GR inhibitor GS-C(O)-NH-Me (Fig. 1; GR inhibitor 1) has been demonstrated to inhibit cell growth (Guest et al., 1992). The objectives of this study were to determine whether GS-C(O)-NH-R (R = p-chlorophenyl) is a metabolite of sulofenur, whether the conjugate is a GR inhibitor, and further, whether the conjugate exhibits cell growth-inhibitory activity by using human colon adenocarcinoma GC/c1 cells, a sulofenur-sensitive cell line. The GC/c1 cell line has been widely employed to explore the anticancer mechanisms of sulofenur and other anticancer diarylsulfonyleureas (Houghton et al., 1990, 1995; Sosinski et al., 1991, 1993; Rush et al., 1992; Phelps et al., 1995). In addition, a glutathione conjugate can be enzymatically converted to a mercapturic acid conjugate (Williams, 1995). Interestingly, the corresponding mercapturic acid conjugate derived from the substituted urea structure [−NH(C(O)-NH-CH₂H₂Cl)] has also been demonstrated to be a GR inhibitor (Fig. 1; GR inhibitor 2) (Davis et al., 1993). Therefore, in addition to glutathione conjugation, we have also investigated the mercapturic acid conjugation pathway of sulofenur. This report describes the identification and quantification of the glutathione conjugate and its corresponding mercapturic acid conjugate from sulofenur-dosed rat bile and urine, respectively, by liquid chromatography/mass spectrometry (LC/MS). We have found that both the glutathione conjugate and mercapturic acid conjugate are GR inhibitors, and both conjugates exhibited cell growth-inhibitory effects against the GC/c1 cell line with potency comparable to sulofenur.

### Materials and Methods

1H and 13C nuclear magnetic resonance spectra were recorded on a Varian 200 MHz NMR spectrometer (Varian, Inc., Palo Alto, CA) and are reported in parts per million. LC/MS was carried out on a Finnigan MAT Navigator HPLC/MS mass detector (Thermo Finnigan, San Jose, CA) interfaced to a SpectraSYSTEM P4000 HPLC system equipped with a SpectraSYSTEM autosampler (San Jose, CA). Mass spectral data were obtained with the Finnigan MAT Navigator HPLC/MS mass detector.

Human colon adenocarcinoma GC/c1 cells were kindly provided by Dr. Peter J. Houghton of St. Jude Children’s Research Hospital (Memphis, TN). Yeast GR, bovine serum albumin (BSA), human recombinant insulin sodium salt, human transferrin, human recombinant epidermal growth factor, GSH, glutathione disulfide, glutathione ethyl ester, N-acetyl-L-cysteine, and reduced nicotinamide adenine dinucleotide phosphate (NADPH) were purchased from Sigma-Aldrich (St. Louis, MO). p-Chlorophenyl isocyanate was obtained from Aldrich Chemical Co. (Milwaukee, WI). RPMI 1640 medium with 25 mM HEPES and fetal bovine serum were purchased from Atlanta Biologicals (Norcross, GA). DispoDialyzers were obtained from Spectra Laboratories (Rancho Dominguez, CA). Male Sprague-Dawley rats (200–225 g) were purchased from the National Cancer Institute (Frederick, MD), and bile duct cannulated male Sprague-Dawley rats (200–225 g) were obtained from Charles River Laboratories (Wilmington, MA). N,N-Bis(2-chloroethyl)-N-nitrosourea (BCNU) was a gift from Bristol-Myers Squibb Pharmaceutical Research Institute (Princeton, NJ). Sulofenur was prepared according to a literature procedure (Howbert et al., 1990) and exhibited NMR and mass spectral characteristics that were fully consistent with the structure. S-(p-Chlorophenylcarbamoyl)glutathione (the glutathione conjugate) and N-acetyl-S-(p-chlorophenylcarbamoyl)cysteine (the mercapturic acid conjugate) were synthesized in this laboratory and described below. All other solvents and chemicals were of HPLC or reagent grade and were used as received.

**Synthesis of S-(p-Chlorophenylcarbamoyl)glutathione.** The glutathione conjugate was synthesized by the reaction of p-chlorophenyl isocyanate with GSH according to a literature procedure (Guan et al., 1994) and purified by semipreparative HPLC [column: Pratisil 10 ODS, 9.5 × 500 mm (Whatman Inc., Clifton, NJ); mobile phase: 50% acetonitrile in water with 0.1% trifluoroacetic acid]. The yield was 13%. 1H NMR (dimethyl sulfoxide-d6): δ 1.87 (m, 2 H, Glu-β', β'), 2.26 (t, J = 7.4 Hz, 2 H, Glu-γ', γ'), 2.94 (dd, J = 13.6, 10.0 Hz, 1 H, Cys-β), 3.46–3.52 (m, 2 H, Cys-β', β''), 3.67 (d, J = 5.7 Hz, 2 H, Gly-α, α'), 4.37–4.45 (m, 1 H, Cys-α), 7.29 (d, J = 8.9 Hz, 2 H, phenyl), 7.46 (d, J = 8.9 Hz, 2 H, phenyl), 8.27 [d, J = 10.0 Hz, 1H, C(OH)Cys], 8.44 [t, J = 5.7 Hz, 1H, C(O)O(NH)Gly], and 10.43 [s, 1 H, C(O)O(NH)phenyl]; 13C NMR (dimethyl sulfoxide-d6): 171.8, 171.2, 170.7, 170.6, 164.9, 138.2, 129.1, 127.3, 120.8, 52.8, 52.7, 42.5, 41.3, 31.5, 26.8. MS: 461 (MH+), 443, 386, 358, 332 (base), 257, 229, 179, 130.

**Synthesis of N-Acetyl-S-(p-Chlorophenylcarbamoyl)cysteine.** The mercapturic acid conjugate was synthesized by the reaction of p-chlorophenyl isocyanate with N-acetyl-L-cysteine. To a 25-ml flask containing N-acetyl-L-cysteine (0.64 g, 3.9 mmol) in tetrahydrofuruan (6 ml) was added p-chlorophenyl isocyanate (0.1 g, 0.65 mmol) at room temperature under argon. The reaction was allowed to stir for an additional 20 min. Tetrahydrofuruan was removed under a reduced pressure, and the residue was suspended in ethyl acetate (2 ml) and filtered. The filtrate was subjected to column chromatographic separation on silica gel 60 (230–400 mesh) with ethyl acetate in hexanes as eluting solvents. The product was obtained in 30% yield (62 mg).

1H NMR (acetonitrile-d6): δ 1.93 (s, 3 H, CH₃), 3.27 (dd, J = 7.8, 14.4 Hz, 1 H, CH₂), 3.56 (dd, J = 4.8, 14.4 Hz, 1 H, CH₂), 4.69 (dd, J = 4.8, 7.8 Hz, 1 H, NCH), 7.33 (d, J = 9.2 Hz, 2 H, phenyl), and 7.60 (d, J = 9.2 Hz, 2H, phenyl). 13C NMR (acetonitrile-d6): 168.8, 167.7, 162.5, 135.6, 126.5, 125.8, 118.3, 50.1, 28.7, and 19.5. MS: 317 (MH+), 299, 164 (base), and 122.

**Identification and Quantification of S-(p-Chlorophenylcarbamoyl)glutathione in Sulofenur-Dosed Rat Bile.** Three bile duct cannulated male Sprague-Dawley rats (200–225 g) were given free access to water and food, except that they were fasted overnight prior to dosing. The rats were anesthetized (i.p.) with sodium phenobarbital (100 mg/kg) and dosed (i.p.) with sulofenur (100 mg/kg) as reported previously (Elhardt, 1991). Rat bile was collected for 5 h in a vial containing a few crystals of vitamin C over dry ice and then stored at −100°C before LC/MS analysis. A control experiment was conducted in parallel in which only the vehicle was administered. Specimens of bile were added with glutathione ethyl ester as an internal standard (1 μg/ml), filtered, and analyzed by LC/MS without further treatment. Quantitation of the analyte was based on the peak area ratio of the analyte to the internal standard.
standard and determined by reference to a standard curve. Standard curves were produced by spiking control bile with the analytes in a range of concentrations: 2, 5, 10, 25, and 50 μg/ml (the glutathione conjugate) or 10, 25, 50, 100, and 200 μg/ml (the mercapturic acid conjugate). The MS employed positive ion electrospray ionization. The HPLC eluate was introduced into the stainless steel electrospray capillary spray held at 2.3 kV. The source and detector voltage were 15 and 650 V, respectively. The low- and high-mass resolutions were set at 12.5 during analysis. Selected ion monitoring was set to simultaneously monitor ions with m/z of 461, 463, and 336, which correspond to the protonated molecular ions [MH⁺] (M + 2H⁺) of the glutathione conjugate and internal standard. The HPLC conditions employed a Polaris C18 column (100 mm × 2.0 mm i.d., 5 μm) (MetaChem Technologies Inc., Torrance, CA), mobile phase A (ammonium acetate, 50 mM, pH 4.5), and mobile phase B (acetoniitrile). At time 0, mobile phase A was pumped iso- cratically for 5 min. Mobile phase B was changed to 25% at 5.1 min and further increased to 60% in 20 min. All flow-rates were 0.2 ml/min. The injection volume was 20 μl.

Identification and Quantification of N-Acetyl-S-(p-Chlorophenylcar- bamoyl)cysteine in Sulofenur-Dosed Rat Urine. Three male Sprague-Dawley rats (200–225 g) in metabolic cages were fasted overnight prior to dosing. The rats were dosed (i.p.) with sulofenur (100 mg/kg) as reported previously (Elhardt, 1991). Rat urine was collected for 24 h in a tube containing a few crystals of vitamin C over dry ice and then stored at −100°C before analysis. A control experiment was conducted in parallel in which only the vehicle was administered. Specimens of urine were added with glutathione ethyl ester as an internal standard (1 μg/ml), filtered, and analyzed by LC/MS as described above, except the selected ion monitoring was set to monitor ions with m/z of 317, 319, and 336, which correspond to the protonated molecular ions [MH⁺] and (M + 2H⁺) of the mercapturic acid conjugate and internal standard.

Inhibition of GR. The inhibition experiment followed a reported procedure for the inhibition of GR by glutathione conjugate analogs (Jochheim and Baillie, 1994). The compound (0.5 mM) was incubated with yeast GR (0.02 unit/ml) in a phosphate buffer (0.1 M, pH 7.4) containing BSA (1 mg/ml) and NADPH (0.2 mM) at 25°C for 30 min. Aliquots were withdrawn for determination of remaining GR activity that was initiated by addition of glutathione disulfide (0.52 mM). GR activity was measured by the initial rates of disappearance of NADPH determined spectrophotometrically at λ = 340 nm.

Inhibition of Human Colon Adenocarcinoma GC/C1 Cells. Anticancer activity evaluation was carried out by determining the proliferation rates of human colon adenocarcinoma GC/c1 cells. Cells were plated at a density of 5000 cells/well (48-well plates) with 6-well replicates/treatment in RPMI 1640 medium containing 0.5% fetal bovine serum. After a 24-h attachment period, the plating medium was removed and replaced with treatment medium, which consisted of RPMI 1640 + 5 μg/ml transferrin + 10 μg/ml insulin + 10 ng/ml epidermal growth factor containing increasing levels of the test compound. Cultures were incubated in a CO2 incubator at 37° C for 6 days, washed with phosphate-buffered saline, and stored frozen. Proliferation was determined by assessing the DNA content of the wells with the Hoechst 33258 fluorescent dye as described by McFarland et al. (1995).

Determination of Protein Binding by Equilibrium Dialysis. BSA solutions (4 g/100 ml) were freshly prepared with a phosphate buffer solution (0.1 M, pH 7.4) containing either the mercapturic acid conjugate or glutathione conjugate (0.1 mM). The solution (0.3 ml) was placed in a 0.5-ml DispoDialyzer with a molecular mass cutoff of 8000 Da and immediately dialyzed against the buffer solution containing the same concentration (0.1 mM) of the mercapturic acid conjugate or glutathione conjugate for 5 h at 25°C with constant shaking. Aliquots (0.2 ml) were withdrawn from each compartment and added with glutathione ethyl ester as an internal standard (1 mg/ml, 20 μl) followed by the addition of acetonitrile (0.4 ml). The sample was vortex-mixed for 1 min and centrifuged (8000 rpm × 5 min). The residue was extracted with acetonitrile (0.2 ml × 2). The combined supernatant was dried under a stream of nitrogen. The residue was then added with phosphate buffer (0.1 M, pH 7.4) to a total volume of 0.2 ml and stored at −80°C before being analyzed by HPLC. The HPLC conditions employed an Adsorbosil C18 column (250 mm × 3.2 mm i.d., 5 μm) (Alltech Associates, Deerfield, IL), mobile phase A (aqueous solution with 0.1% trifluoroacetic acid), and mobile phase B (acetonitrile). The detection of analytes was performed at 220 nm.

Identification and Quantification of the Glutathione Conjugate and Mercapturic Acid Conjugate. Following administration of sulofenur to rats by i.p. injection, a targeted search was made for the glutathione conjugate in rat bile and the mercapturic acid conjugate in rat urine. This was achieved with the aid of LC/MS. Selected ion monitoring was employed to detect the glutathione conjugate and mercapturic acid conjugate. Since both conjugates contain one atom of chlorine, MH⁺ (m/z 461 for the glutathione conjugate and 317 for the mercapturic acid conjugate) and (M + 2)H⁺ (m/z 463 for the glutathione conjugate and 319 for the mercapturic acid conjugate) were simultaneously monitored for each conjugate. Identification of the metabolites was based on the fact that the metabolites exhibited LC/MS properties identical to the corresponding authentic conjugates prepared by synthesis and was further supported by the intensity ratio of MH⁺/(M + 2)H⁺, consistent with the isotope ratio of 35Cl/37Cl (−3:1). Fig. 3a is a representative LC/MS chromatogram derived from a sulofenur-dosed rat bile sample, and Fig. 3b is one derived from an authentic glutathione conjugate. A peak with identical LC/MS properties as the authentic glutathione conjugate is present in the sulofenur-dosed rat urine sample (Fig. 3, a and b). Similarly, a peak identical to the authentic mercapturic acid conjugate is present in the sulofenur-dosed rat urine sample (data not shown). The corresponding peaks for the glutathione conjugate and mercapturic acid conjugate were not observed in the control samples that were obtained from the vehicle only dosed rats. The intensity ratios of MH⁺/(M + 2)H⁺ were 2.08:1 and 2.95:1 for the glutathione conjugate and mercapturic acid conjugate, respectively. These values were consistent with the intensity ratios obtained from the synthetic standards (2.17:1 and 2.87:1 for the glutathione conjugate and mercapturic acid conjugate). Based on these data, we conclude that the glutathione conjugate and mercapturic acid conjugate were the metabolites of sulofenur. Quantitation of the metabolites was based on the peak area ratio of the metabolite to the internal standard and determined by reference to a standard curve. The linear correlation coefficients were 0.9994 and 0.9873 for the glutathione conjugate and mercapturic acid conjugate, respectively. Both conjugates appear to be minor metabolites. The fraction of the dose of sulofenur as the glutathione conjugate over 5 h was 0.12 ±
TABLE 1

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<thead>
<tr>
<th>Compounds</th>
<th>IC₅₀ (µM)</th>
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<tr>
<td>Glutathione conjugate</td>
<td>3.0 ± 2.3</td>
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<tr>
<td>Mercapturic acid conjugate</td>
<td>5.3 ± 0.5</td>
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<tr>
<td>Sulofenur</td>
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*Result from one experiment with six replications.

Inhibition of GR by the Glutathione Conjugate and Mercapturic Acid Conjugate. When the glutathione conjugate and mercapturic acid conjugate were incubated with yeast GR, both exhibited inhibitory activity against the enzyme. To evaluate the inhibitory potency of these two conjugates, we added BCNU, which is the most commonly used GR inhibitor (FitzGerald et al., 1991), as a positive reference. The remaining GR activity in the presence of the glutathione conjugate, BCNU, and the mercapturic acid conjugate was 13.8 ± 2.1, 22.4 ± 2.2, and 77.2 ± 12.3%, respectively. These results indicated that the glutathione conjugate is a more potent GR inhibitor than BCNU whereas the mercapturic acid conjugate is a weaker inhibitor than BCNU.

Inhibition of Human Colon Adenocarcinoma GC/c1 Cells. Incubation of human colon adenocarcinoma GC/c1 cells with the glutathione conjugate and mercapturic acid conjugate revealed that both conjugates significantly inhibited cancer cell growth. The IC₅₀ values that were derived from the dose-response curves for the glutathione conjugate and mercapturic acid conjugate are included in Table 1. IC₅₀ is the concentration producing 50% inhibition of cell growth. For reference purposes, we also determined the IC₅₀ value of sulofenur. The IC₅₀ value of sulofenur obtained in this study was 2 µM, compared with a literature value (0.51 µM) that was obtained under similar conditions (Sosinski et al., 1993). Table 1 shows that the glutathione conjugate and mercapturic acid conjugate inhibited activity against human colon adenocarcinoma GC/c1 cells comparable to that of sulofenur.

Determination of Protein Binding by Equilibrium Dialysis. Determination of protein binding by equilibrium dialysis revealed that the glutathione conjugate and mercapturic acid conjugate exhibited inhibitory activity against human colon adenocarcinoma GC/c1 cells comparable to that of sulofenur.

Discussion

Diarylsulfonylureas, including sulofenur, have been developed as a novel class of anticancer agents with a broad spectrum of activity in several solid tumor models but as yet an unidentified mechanism of action (Toth et al., 1997). Sulofenur has undergone extensive clinical trials based on its impressive preclinical activity. However, the clinical response was unsatisfactory because of methemoglobinemia, which is associated with a higher than in vitro dose required to overcome the high protein binding problem of the drug. Elucidation of the metabolic pathways of sulofenur may help to understand the anticancer mechanism of the drug and may provide useful information to reduce side effects. Since sulofenur has a urea structural feature of [-N(H or R')-C(O)-NH-R], a structural feature that has been demonstrated to give a glutathione conjugate and its degradation product mercapturic acid conjugate, it is reasonable to expect that sulofenur may produce the corresponding glutathione conjugate and mercapturic acid conjugate. We have demonstrated that sulofenur, like other substituted urea analogs, is metabolically converted to a glutathione conjugate and mercapturic acid conjugate. The significance of the formation of these two conjugates is that these conjugates have been shown in this study to be GR inhibitors. We did not fully characterize the enzyme inhibitory kinetics of the glutathione conjugate due to the limited amount of the compound available. An enzyme inhibitory kinetic study of the mercapturic acid conjugate indicated that the inhibition was irreversible (data not shown). The irreversibility of the inhibition was further confirmed by the fact that no enzyme activity was observed after extensive dialysis of the inhibited enzyme (data not shown). These observations were consistent with the inhibitory properties of their corresponding glutathione conjugate analogs derived from the substituted ureas discussed earlier (Davis et al., 1993; Guan et al., 1994, 1999; Jochheim and Baillie, 1994). Furthermore, our study has revealed that both conjugates exhibited inhibitory activity comparable to the parent drug against the human colon adenocarcinoma GC/c1 cell line, a cell line that has been widely employed to explore the anticancer mechanism(s) of sulofenur and other anticancer diarylsulfonylureas (Houghton et al., 1990, 1995; Sosinski et al., 1991, 1993; Rush et al., 1992; Phelps et al., 1995).

It is not clear at this point whether the inhibition of GR contributes to the cancer cell growth-inhibitory activity of these two conjugates. It is known that inhibition of GR can lead to the depletion of GSH (Kassahun et al., 1994). Depletion of intracellular GSH has been demonstrated to be an effective approach in decreasing cellular proliferation rates and cancer cell growth (Terradze et al., 1993; Lasso de la Vega et al., 1994; Estrela et al., 1995). Recently, GSH depletion has been shown to effectively cause apoptosis in prostate carcinoma cells (Coffey et al., 2000). We did not explore the effect of these conjugates on intracellular GSH. However, S-(N-methylcarbamoyl)cysteine (Fig. 1) has been demonstrated to deplete intracellular GSH (Kassahun et al., 1994). S-(N-methylcarbamoyl)cysteine is a weaker GR inhibitor than BCNU (Jochheim and Baillie, 1994). Our enzyme inhibitory study has shown that the glutathione conjugate is a more potent GR inhibitor than BCNU, whereas the mercapturic acid conjugate is a less potent GR inhibitor than BCNU. Therefore, it is likely that these two conjugates may reduce intracellular GSH levels. Further study needs to be conducted to investigate the role of GR inhibition in the anticancer activity of these two conjugates.

The contributions of these two conjugates in the anticancer activity of sulofenur also remain to be defined. One may argue that since these are minor metabolites, their contributions to the anticancer activity of the parent drug may not be significant. However, it is noteworthy that although these two conjugates only account for less than 3% of the total dose, this amount may be significant enough in contributing to the anticancer activity of sulofenur considering the high protein binding property (>99.9%) (Elhlhardt, 1991).
the anticancer activity of sulofenur, further examination with other sulofenur-sensitive cell lines needs to be conducted. However, it is reasonable to suggest that these conjugates may be partially responsible for the in vivo anticancer activity of sulofenur.

Overall, this study has provided evidence that sulofenur undergoes metabolic conversion to form a glutathione conjugate and mercapturic acid conjugate. These conjugates are GR inhibitors and also exhibit significant inhibitory activity against one of the sulofenur-sensitive human cancer cell lines. The role of GR inhibition caused by these conjugates in cancer cell growth inhibition and the contribution of these conjugates to the anticancer activity of the parent drug remains to be further examined. In addition, the revelation of these conjugates with cancer cell growth-inhibitory activity may lead to the development of a novel class of anticancer agents. An investigation of the anticancer spectrum of these conjugates and their structural analogs is underway.

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References


Houghton PJ, Cheshire PJ, Myers L, Lutz L, Toth J, Grindey GB, and Houghton JA (1995) Efficacy of sulofenur and a second generation diarylsulfonylurea, N-[5-(2,3-dihydrobenzofu-

caryl)sulfamoyl]-N′-(3,4-dichlorophenyl)urea (LY295503), against colon adenocarcinoma xenografts. Anticancer Drugs 6:317–323.

Houghton PJ and Houghton JA (1996) Antitumor diarylsulfonylureas: novel agents with unful-


Phillips PC, Jain PT, Berezesky IK, Boder GD, and Trump BF (1995) Sulofenur cytotoxicity and changes in cytosolic calcium and mitochondrial membrane potential in human colon adeno-


Sosinski J, Chapin C, Thakur JH, and Houghton PJ (1991) Inhibition of extracellular pH on the accumulation and cytotoxicity of N-(4-methylphenyl)sulfonyl-N′-(4-chlorophenyl)urea in hu-

man cell lines. Cancer Commun 3:373–381.

Sosinski J, Thakur JH, Germain GS, Harwood FC, and Houghton PJ (1993) Proliferation-

