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Acute effects of novel selenazolidines on murine chemoprotective enzymes

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Abstract

Novel selenazolidines, designed as L-selenocysteine prodrugs and potential cancer chemopreventive agents, were examined for their ability to affect the transcription of murine hepatic chemoprotective enzymes. Compounds investigated were selenazolidine-4(*R*)-carboxylic acid (SCA) and six 2-substituted derivatives that cover a $C\log P$ range of −0.512 to −3.062. Their biological effects were compared with those of L-selenocystine. Gene transcripts were examined 24 h after a single dose, administered i.p. and i.g., and covered a range of chemoprotective enzymes; alpha, mu and pi class glutathione transferases (Gsts), UDP-glucuronosyltransferases (Ugts) 1a1, 1a6, 1a9, and 2b5, glutathione peroxidase 1 (Gpx), thioredoxin reductase (Tr), NAD(P)H-quinone oxidoreductase 1 (Nqo), and microsomal epoxide hydrolase (Meh). When given i.g., 2-butyl SCA (BSCA) resulted in elevations in alpha, mu and pi class Gsts, Ugt1a6, Tr, and Gpx, and 2-phenyl SCA (PhSCA) elevated GstP, Ugt1a9, Tr, Gpx (3 kb), and Meh. Other derivatives with $C\log P$ values both lower [2-(2′-hydroxy)phenyl SCA (PhOHSCA) and 2-methyl SCA (MSCA)] and higher [2-cyclohexyl SCA (ChSCA) and 2-oxo SCA (OSCA)] than BSCA and PhSCA elevated far fewer transcripts; PhOHSCA (Ugt1a1, Gpx), MSCA (Ugt1a1, Meh), ChSCA (Ugt1a1, Ugt1a9), and OSCA (Ugt1a6, Ugt1a9, GstM). When given i.p., the most pervasive transcript changes were parallel increases in Nqo and Tr transcripts which occurred with BSCA, PhSCA, MSCA, and OSCA. PhSCA also increased GstP, and PhOHSCA increased Ugt1a1 and Ugt1a6 levels. Unique among the compounds, PhSCA reduced the transcript levels of GstA, and the 1.6 kb transcript of Gpx although only when given i.p. Neither L-selenocystine nor SCA affected the level of any transcript and no compound altered the amount of Ugt2b5 mRNA. Despite chemical similarity and common ability to potentially serve as a source of L-selenocysteine, each selenazolidine compound appeared to elicit a unique pattern of mRNA responses and by either route of administration, there was no correlation between the magnitude of response of any gene and the calculated $C\log P$ values of the organoselenium compounds.
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Keywords: Selenium; Selenocysteine prodrugs; Selenazolidines; Mouse; mRNA; Thioredoxin reductase; Glutathione peroxidase; NAD(P)H-quinone oxidoreductase; Glutathione transferases; UDP-glucuronosyltransferases; Microsomal epoxide hydrolase

1. Introduction

For more than four decades selenium has been categorized as an essential mammalian micronutrient. Although selenium and selenium-containing compounds provide protection against many degenerative conditions, including cancer, the mechanism or mechanisms

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by which this occurs continues to require investigation. In chemoprevention against cancer, an effect related to the maintenance or enhancement of "protective" enzymes is commonly invoked. Protective enzymes include the selenoproteins glutathione peroxidase (GPx) and thioredoxin reductase (TR), quinone oxidoreductase (NQO), as well as UDP-glucuronosyltransferases (UGTs), microsomal epoxide hydrolase (mEH) and glutathione transferases (GSTs). All are involved in either sequestering reactive oxygen species and reactive electrophilic metabolites or maintaining cellular components in their appropriate redox status.

In the study of selenium and selenium compound efficacy in chemoprevention, naturally occurring compounds have not always shown convincing and comprehensive activity. Inorganic selenite, in a variety of dosing regimens but centered around a selenium dose level of around 5 ppm, was not effective against 7,12-dimethylbenz[*a*]anthracene (DMBA)-induced rat mammary tumors [1], 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK)-induced mouse lung tumors [2,3] or azaserine-induced rat pancreatic and hepatic neoplasms [4]. However, selenite at similar dose levels, was effective against DMBA-induced mammary tumors in mice [5] and rats [6–8], although only weakly so [9]. Of the selenium-containing amino acids and their derivatives, selenocystine at selenium dose levels of 2 and 3 ppm was not effective against DMBA-induced mammary tumors in mice [5], and only weakly effective [9], or not effective [10] in rat, whereas in mice, and at 15 ppm, it was effective for NNK-induced lung tumors [3]. Selenomethionine at selenium dose levels of 2–4 ppm was not effective against DMBA-induced mammary tumors in mice [5] and only weakly effective against DMBA-induced mammary tumors in rat [9] and NNK-induced lung tumors in mice [3]. Modified seleno-amino acids, Se-methyl-, Se-propyl-, and Se-allylselenocysteines were effective against DMBA and *N*-methyl-*N*-nitrosourea (MNU)-induced mammary tumors in rat [10–12] but Se-methylselenocysteine at similar dose levels (3 ppm) was not effective against NNK-induced lung tumors in mice [3].

In the search for more consistently efficacious compounds, several organoselenium compounds particularly organoselenocyanates have been created and evaluated, although again with a variety of dosing regimens that makes direct efficacy comparisons difficult. In general, these compounds have been investigated at much higher selenium dose levels (up to 40 ppm) than was possible with inorganic selenite. Benzylselenocyanate was effective against benzo[*a*]pyrene-induced mouse forestomach tumors [13], azoxymethane-induced rat liver tumors [14] and DMBA-induced rat mammary tumors [1]. 1,4-Phenylenebis(methylene) selenocyanate (*p*-XSC) also had wide ranging efficacy against tumors induced by a variety of carcinogens in many tissues; in rat colon after azoxymethane [15], in rat mammary tissue after DMBA [16], in mouse lung after NNK [2,17–20], and in rat tongue after 4-nitroquinoline-1-oxide [21]. It was also effective against spontaneous familial adenomatous polyposis development in APC(min) mice [22]. The di-glutathione conjugate of *p*-XSC was also effective against azoxymethane-induced tumors in rat colon [23]. Aliphatic rather than aromatic selenocyanates have also proved effective against DMBA-induced rat mammary tumors [24].

Organoselenium compounds in addition to selenocyanates have been investigated, mostly with rat mammary tumor models and have met with varying degrees of efficacy. Methylphenylselenide, *p*-xylylbis(methylselenide), triphenylselenonium chloride, and diphenyl selenide were investigated against MNU-induced tumors [25], and triphenylselenonium chloride and diphenyl selenide against DMBA-induced tumors [26,27]. Early studies with *p*-methoxybenzeneselenol had found it to be effective against benzo[*a*]pyrene-induced mouse forestomach tumors [13] and azoxymethane-induced rat liver tumors [28].

Of all the organoselenium compounds developed and evaluated, few have been examined for the changes they elicit in chemoprotective enzymes, and where such studies have been undertaken, most have examined activities of only one or two, most commonly including GSTs. Most of these changes were investigated after several months of organoselenium compound administration, with only a couple of studies evaluating changes after a week, and none after just one or a few days. Recently, elevated GST activity was reported in mouse liver, skin, and colon following diphenylmethylselenocyanate administration [29,30]. In earlier studies, GSTM activity was increased in colon mucosa of azoxymethane-treated rats after 10 weeks of dietary exposure to the glutathione conjugate of benzylselenocyanate [31]. With long-term administration of *p*-XSC to otherwise naïve animals, no elevation of rat liver GST (1-chloro-2,4-dinitrobenzene, CDNB) activity was seen [21]. However, in a 1-week study with lower *p*-XSC concentrations, increased GST (CDNB) activity in liver, lung and kidney but not colon or mammary tissue was reported [32]. Utilizing class-selective substrates, GSTA activity was increased only in lung, and GSTP activity in lung and colon [32]. No GST class-selective substrate activity was statistically increased in liver. In a similar study in mice,

GST (CDNB) activity, as well as GSTP and GSTM (not GSTA) class-selective activities were elevated in lung and liver [19]. In these latter two studies, Se-dependent-GPx activity was elevated in the rat colon but not liver or lung of rat or mouse. An increase in rat colon mucosal GPx activity by dietary *p*-XSC had previously been shown [15]. Another organoselenium compound, triphenylselenonium chloride, did not increase rat liver GPx activity [27]. Neither triphenylselenonium chloride [27], nor Se-methylselenocysteine [33] influenced rat hepatic TR activity. Of two other protective enzyme activities investigated, NQO activity was increased [21] and UGT (4-nitrophenol) activity was slightly elevated [32] by *p*-XSC in rat liver. In mice, neither lung nor liver UGT activity was significantly affected by *p*-XSC [19].

We have pursued an L-selenocysteine prodrug approach to delivering supranutritional levels of bioavailable selenium. Several selenazolidines have been created that by enzymatic or hydrolytic cleavage are designed to slowly release L-selenocysteine [34]. Of three such prodrugs recently tested for chemopreventive activity against NNK-induced lung tumors in a mouse model, 2-oxoselenazolidine-4-carboxylic acid at 15 ppm selenium in the diet reduced the number of lung tumors by 37.5% [3]. Chemoprotection studies are not only costly but require an extensive time frame to complete so there remains a compelling need for short-term assays to prescreen newly synthesized compounds. Towards this end, and with the mode of action of some selenium compounds possibly linked to their ability to affect protective enzymes, we have examined the effect of a single dose of seven selenazolidines on the levels of the transcription products (mRNAs) of the genes of the protective enzymes. Since selenium-related chemoprevention against tumors can occur in many tissues in animals models, including liver, we screened for these enzyme transcript changes in liver, a readily available and responsive tissue with a relatively high proportion of a single cell type.

2. Materials and methods

2.1. Chemicals

SCA, MSCA, and OSCA were synthesized as previously described [34,35]. The remaining 2-substituted selenazolidines were prepared by the condensation of L-selenocysteine (Acros Organics, Morris Plains, NJ) and the appropriate carbonyl compound. The structures of the target compounds were verified on the basis of elemental and spectroscopic methods of analysis. The calculated $C\log P$ values are shown in Table 1.

2.2. Animal treatment and biological sample preparation

Adult male CF-1 mice (25–35 g) from Charles River Laboratories were maintained in humidity- and temperature-controlled environment on a 12-h light/dark cycle with free access to food (Harlan-Teklad Laboratory Diet 8640; Se content 0.026 ppm) and water. Compounds were administered by gavage (i.g.) or intraperitoneal injection (i.p.) at selenium doses of 0.55 mg/kg (L-selenocystine) or 1.1 mg/kg (selenazolidines), a dose comparable to the amount of selenium ingested daily when the selenazolidines are included in the diet at 15 ppm as in the chemoprevention study [3]. SCA, MSCA, OSCA, and selenocystine were dissolved and administered in 0.2 ml of water, BSCA, ChSCA, PhSCA, and PhOHSCA in 0.2 ml of corn oil. All animal procedures were approved by the University of Utah Animal Care and Use Committee and were conducted in concordance with NIH guidelines for the humane care of laboratory animals. Animals were sacrificed 24 h after the single dose, a blood sample was collected on ice for immediate serum preparation, and the livers were snap-cooled by perfusion in situ (via the hepatic portal vein) with ice-cold normal saline.

Table 1
Selenazolidine L-selenocysteine prodrugs

Selenazolidine	Abbreviation	2-Substituent	$C\log P$
Selenazolidine-4(*R*)-carboxylic acid	SCA[a]	H	−3.062
2-Methylselenazolidine-4(*R*)-carboxylic acid	MSCA[a]	CH_3	−2.543
2-Oxoselenazolidine-4(*R*)-carboxylic acid	OSCA[a]	=O	−0.529
2-Butylselenazolidine-4(*R*)-carboxylic acid	BSCA[b]	C_4H_9	−0.956
2-Phenylselenazolidine-4(*R*)-carboxylic acid	PhSCA[b]	C_6H_5	−1.884
2-Cyclohexylselenazolidine-4(*R*)-carboxylic acid	ChSCA[b]	cyclo-C_6H_{11}	−0.512
2-(2′-Hydroxyphenyl)-selenazolidine-4(*R*)-carboxylic acid	PhOHSCA[b]	2′-(OH)C_6H_4	−2.551

[a] Administered to mice in water.
[b] Administered to mice in corn oil.

A 100 mg sample of liver was removed, homogenized in 2 ml of TRIzol solution (Invitrogen; Carlsbad, CA) and frozen at −80 °C for later RNA isolation. In the study investigating any GST activity changes in animals receiving the selenazolidines administered in corn oil, livers, with the gall bladder carefully dissected away, were homogenized in 0.25 M sucrose and cytosolic fractions (105,000 × *g* for 60 min) prepared by differential centrifugation.

2.3. Hepatotoxicity evaluation

Serum alanine aminotransferase (sALT) [glutamate-pyruvate transaminase] activity was determined using a coupled reaction in which the serum dependent absorbance change of NADH oxidation was monitored at 340 nm in the presence of optimized concentrations of L-alanine, α-ketoglutarate and purified lactic dehydrogenase enzyme [36].

2.4. mRNA quantification

Hepatic mRNA levels were determined by Northern blotting of 20 μg of total RNA isolated by TRIzol extraction. Gel electrophoresis, nucleic acid transfer to membranes and ^{32}P probe labeling were all performed as described previously [37]. The cDNA fragments used as probes for northern blotting were as documented in Table 2. The cDNA probes for the alpha and mu class glutathione transferases were each designed to capture as many members within a class as possible by utilizing the regions with highest homology within the genes of the class, while having the greatest dissimilarity for members of other classes. Hybridized blots were washed (high stringency) as described previously [38]. Washed blots were exposed to autoradiographic film with an intensifying screen at −70 °C, and when sufficient band density for optimal quantification had developed, the density of the major discrete band or bands (two for Gpx1 and GstP, possibly representing different transcripts or enzymes) was determined by scanning densitometry (Molecular Analysis Software; Bio-Rad, Hercules, CA). All mRNA bands were normalized to the same-sample cyclophilin mRNA band. Results are expressed as fold change from the appropriate control animals.

2.5. Glutathione transferase activity

The protein concentration of the cytosolic fraction was determined by the method of Lowry et al. [39], and the glutathione transferase activity towards 1-chloro-2,4-dinitrobenzene determined spectrophotometrically in the presence of saturating reduced glutathione as outlined by Habig and Jakoby [40].

2.6. Statistical analysis of data

Results are expressed as the mean ± S.E.M. Fold changes elicited by BSCA, ChSCA, PhSCA, and PhOHSCA were compared to vehicle (corn oil)-treated controls and SCA, MSCA, OSCA, and selenocystine were compared to untreated animals. Treatment group size was 3 for mice receiving SCA, MSCA, OSCA, and selenocystine, and 4 for mice receiving corn oil. Statistical analyses were performed using ANOVA, followed by Fisher's protected least significant difference multiple range test. Differences were considered significant at *P* values of <0.05.

Table 2

Probe	Sequence	Homology
Ugt1a1	2-870 (rat) *UGT1A1* (U20551)	90% (mouse) *Ugt1a1* 42-939 (NM_201645), 52% (mouse) *Ugt1a6* (U09930), 52% (mouse) *Ugt1a9* (BC026561), <50% (mouse) *Ugt2b5* (X06358)
Ugt1a6	9-765 (U09930)	
Ugt1a9	1-750 (BC026561)	
Ugt2b5	1-804 (X06358)	
Meh,	107-1531 (rat) *EPHX1* (M26125)	93% (mouse) *Ephx1* 70-1440 (NM_010145), <50% (mouse) *Ephx2* (BC015087)
Gpx	1-670 *Gpx1* (NM_008160)	71% *Gpx2* (NM_030677), 65% *Gpx3* (NM_008161), 58% *Gpx4* (NM_008162), 65% *Gpx5* (NM_010343)
Nqo	1-1480 *Nqor1* (NM_008706)	<50% *Nqor2* (NM_020282)
Tr	159-539 *Txnrd1* (AB027565)	61% *Txnrd2* (BC052758), 68% *Txnrd3* (NM_153162)
GstA	1-700 *Gsta2* (NM_008182)	97% *Gsta1* (NM_00818), 76% *Gsta3* (NM_010356), 66% *Gsta4* (NM_010357), <50% *Gstm1* and *Gstp1*
GstM	200-784 *Gstm1* (NM_010358)	87% *Gstm2* (NM_008183), 91% *Gstm3* (NM_010359), 66% *Gstm4* (NM_026764), 86% *Gstm5* (J04696), 85% *Gstm6* (NM_008184), <50% *Gstp1*
GstP	185-622 *Gstp1*[a] (NM_013541)	98%*Gstp2* (NM_181796)

[a] The GstP probe was a gift from Dr. C. Roland Wolf, University of Dundee, Scotland.

3. Results

The sALT values determined 24 h after a single dose indicated that none of the investigated compounds by either route of administration caused any acute hepatotoxicity (Table 3). The absence of hepatotoxicity is important in the subsequent interpretation of any observed changes in mRNAs as not being a consequence of overt liver cell damage or death.

The i.g. route of administration produced the greater number of significant changes in mRNA levels as compared to the i.p. route. This is most evident among the microsomal enzyme mRNA responses (Ugts and Meh; Figs. 1 and 2, respectively), where with the exception of PhOHSCA, all statistically significant mRNA elevations occurred following i.g. administration. By this route, MSCA and ChSCA induced Ugt1a1, OSCA and BSCA induced Ugt1a6, and OSCA, ChSCA and PhSCA induced Ugt1a9. PhOHSCA induced Ugt1a1, but in addition, increased this and the Ugt1a6 transcript following i.p. administration. In contrast to the inducibility of the Ugt1a family, none of the compounds by either route affected Ugt2b5. Significant elevations in Meh mRNA were confined to i.g. administration, and this occurred with MSCA and PhSCA (Fig. 2). A similar situation existed with Gpx where elevations (seen only for the larger (i.e. 3 kb) of two transcripts) were also restricted to i.g. administration (Fig. 3). For this gene, the elevations were seen with BSCA, PhSCA and PhOHSCA and all were very similar at approximately two-fold.

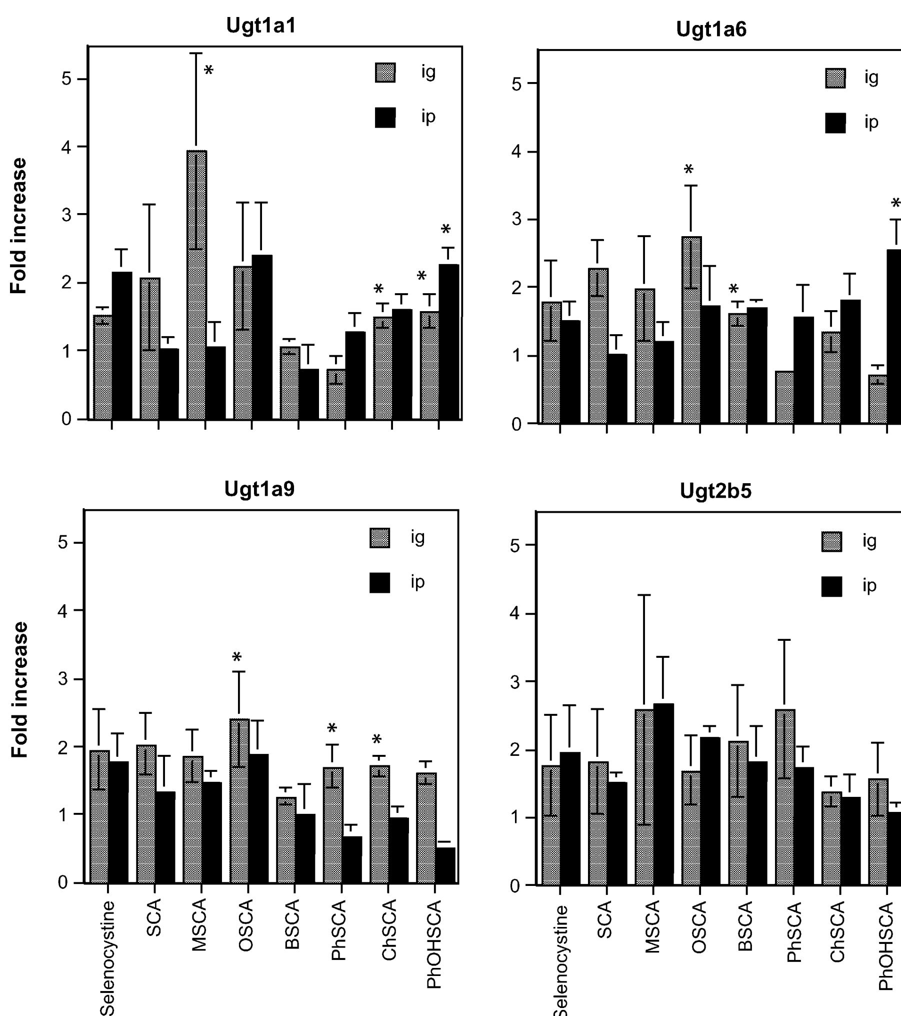

Fig. 1. Effect of selenazolidine L-selenocysteine prodrugs on hepatic mRNAs of UDP-glucuronosyltransferases. Selenazolidine compounds were administered at 1.1 mg Se/kg; L-selenocystine was administered at 0.55 mg Se/kg. SCA, MSCA, OSCA, and selenocystine were compared with the untreated mice. BSCA, PhSCA, ChSCA, and PhOHSCA were compared with mice receiving corn oil vehicle. *Significantly different from the appropriate control, $P < 0.05$.

Table 3
Effect of selenazolidine L-selenocysteine prodrugs on a hepatotoxicity marker enzyme (glutamate-pyruvate transaminase, sALT) in serum

Compound	sALT (mU/ml)	
	i.g.	i.p.
Selenocystine	43.87 ± 4.54	54.70 ± 7.45
SCA	45.78 ± 2.24	39.15 ± 4.25
MSCA	41.02 ± 3.03	31.70 ± 5.12
OSCA	38.78 ± 5.08	35.24 ± 3.51
BSCA	27.09 ± 3.27	24.63 ± 2.84
PhSCA	34.61 ± 2.58	27.70 ± 4.54
ChSCA	23.45 ± 1.41	21.89 ± 0.77
PhOHSCA	26.05 ± 3.41	23.18 ± 2.41

Selenazolidines were administered at 1.1 mg Se/kg, L-selenocystine was administered at 0.55 mg Se/kg. No value showed a statistically significant change from its appropriate control; untreated animals for selenocysteine, SCA, MSCA, and OSCA, corn oil-treated animals for BSCA, ChSCA, PhSCA, and PhOHSCA.

PhSCA given i.p. produced the lone significant change in the 1.6 kb Gpx transcript, a decrease. Although the methodology employed here does not allow definitive assignment of the two bands on the Gpx northern blot to either different enzymes or to alternative transcripts of the same gene, the former is less likely since there is only 58–71% sequence homology for other *Gpx* genes

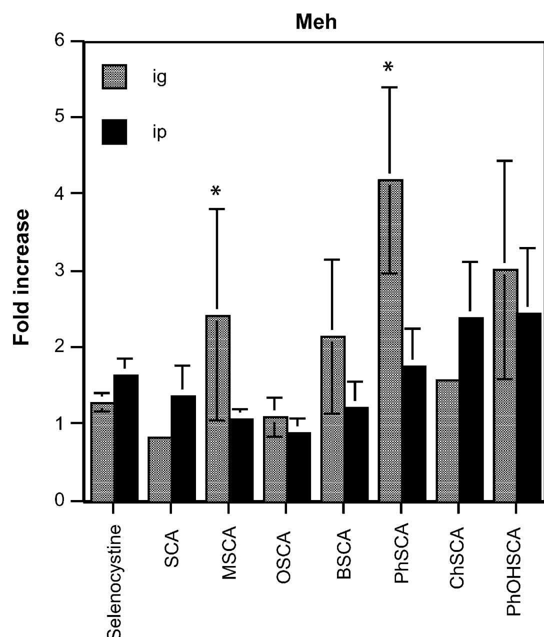

Fig. 2. Effect of selenazolidine L-selenocysteine prodrugs on liver microsomal epoxide hydrolase mRNA. Selenazolidine compounds were administered at 1.1 mg Se/kg; L-selenocystine was administered at 0.55 mg Se/kg. SCA, MSCA, OSCA, and selenocystine were compared with the untreated animals. BSCA, PhSCA, ChSCA, and PhOHSCA were compared with the corn oil-treated animals. *Significantly different from the appropriate control, $P < 0.05$.

and the northern blots were subject to high stringency wash conditions.

The predominance of significant elevations with i.g. administration can be similarly observed with the Gst responses, where OSCA, BSCA and PhSCA produced significant changes but only with PhSCA were changes also seen with i.p. administration (Fig. 4). The responses to PhSCA given i.p. were as robust as the changes when given i.g.; approximately five-fold increases in both the 2.4 and 1.2 kb GstP transcripts. The two bands could either be alternative transcripts or different enzymes, since the northern blot wash conditions would not differentiate gene transcripts with the 98% homology that exists between the *Gstp1* probe sequence and *Gstp2*. In contrast to PhSCA, BSCA (i.g.) also increased the mRNA levels of GstA and GstM. GstA mRNA levels were affected by one other compound, PhSCA, but for this compound there was a reduction in the level of the GstA transcript. Among the selenium compounds administered in aqueous vehicle (SCA, MSCA, OSCA, selenocystine), only OSCA which increased the GstM mRNA when given i.g., affected any of the Gst enzymes.

Whether the mRNA changes had resulted in elevations in GST activity during the 24 h period was evaluated for the compounds administered in corn oil (Table 4), the compounds eliciting the largest, and all but one of the significant, mRNA changes (Fig. 4). No compound showed an elevation in GST activity. The only statistically significant change in activity was an anomalous decrease by ChSCA given i.g.

The mRNA responses of two genes that were contrary to the general dominance of the i.g. effects over i.p. effects were with Nqo and Tr (Fig. 3). Not only were most significant elevations seen with i.p. administration (MSCA, OSCA, BSCA, PhSCA), but both genes were elevated by the same compounds. Although parallel, the

Table 4
Effect of selenazolidine L-selenocysteine prodrugs on hepatic glutathione transferase activity

Compound	GST (1-chloro-2,4-dinitrobenzene) activity (nmol/mg cytosolic protein/min)	
	i.g.	i.p.
Corn oil (vehicle)	4884 ± 414	4477 ± 320
BSCA	5555 ± 420	5401 ± 533
PhSCA	5451 ± 671	5178 ± 267
ChSCA	3508 ± 225*	4407 ± 331
PhOHSCA	4033 ± 197	3841 ± 202

Selenazolidines were administered at 1.1 mg Se/kg in corn oil vehicle.
* Significantly different ($P < 0.05$) from same-route corn oil vehicle control.

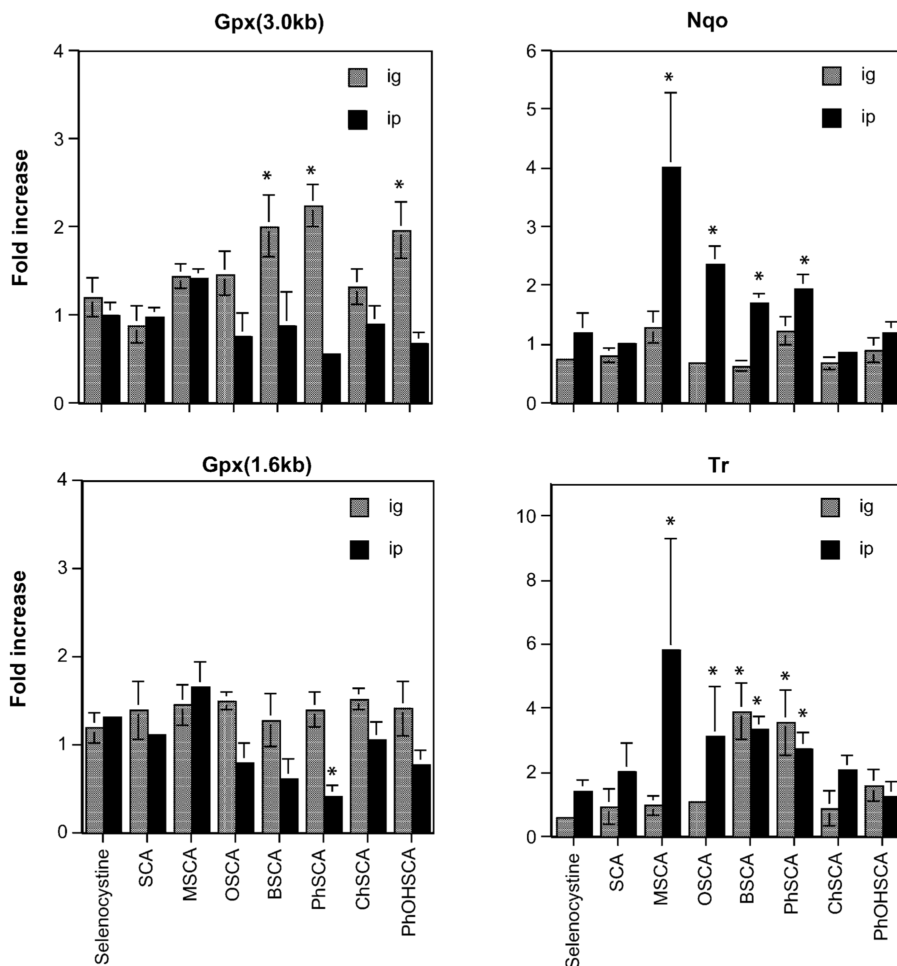

Fig. 3. Effect of selenazolidine L-selenocysteine prodrugs on hepatic mRNAs of glutathione peroxidase, NAD(P)H-quinone oxidoreductase and thioredoxin reductase. Selenazolidine compounds were administered at 1.1 mg Se/kg; L-selenocystine was administered at 0.55 mg Se/kg. SCA, MSCA, OSCA, and selenocystine were compared with the untreated animals. BSCA, PhSCA, ChSCA, and PhOHSCA were compared with the corn oil-treated animals. *Significantly different from the appropriate control, $P < 0.05$.

magnitude of the increase was greater for Tr than for Nqo. In addition to the effects seen with i.p. administration, Tr mRNA but not Nqo mRNA was also elevated by the i.g. administration of BSCA and PhSCA.

4. Discussion

The ratio of maximum tolerable dose (MTD):dose producing 50% reduction (ED_{50}) of total tumor number has frequently been low for inorganic selenium compounds and naturally occurring selenium containing amino acids, and this has fueled searches for compounds with more favorable properties. Dominant among them have been modified selenium amino acids [12], organoselenides [25], and organoselenocyanates among which *p*-XSC has been most studied. This compound showed efficacy against a variety of chemically induced tumors in several organs of rat and mouse [2,15,16,21]. Surprisingly, given the expense of mounting tumor prevention studies, and their duration, few if any of the compounds developed have been analyzed for their acute effects at the level of gene expression in animals. Gene expression has most often been deduced from changes in tissue enzyme activities determined following chronic administration, usually in the diet. Enzyme activities were not universally determined in the present single dose study because with other inducing agents of protective enzymes in vivo, the maximal changes in hepatic mRNA levels of the genes occurred around the 18-h period [41] and it was anticipated that any translation-dependent changes would not be sufficiently robust until sometime beyond the 24-h period at which the animals were sacrificed. This assumption was borne out by limited studies

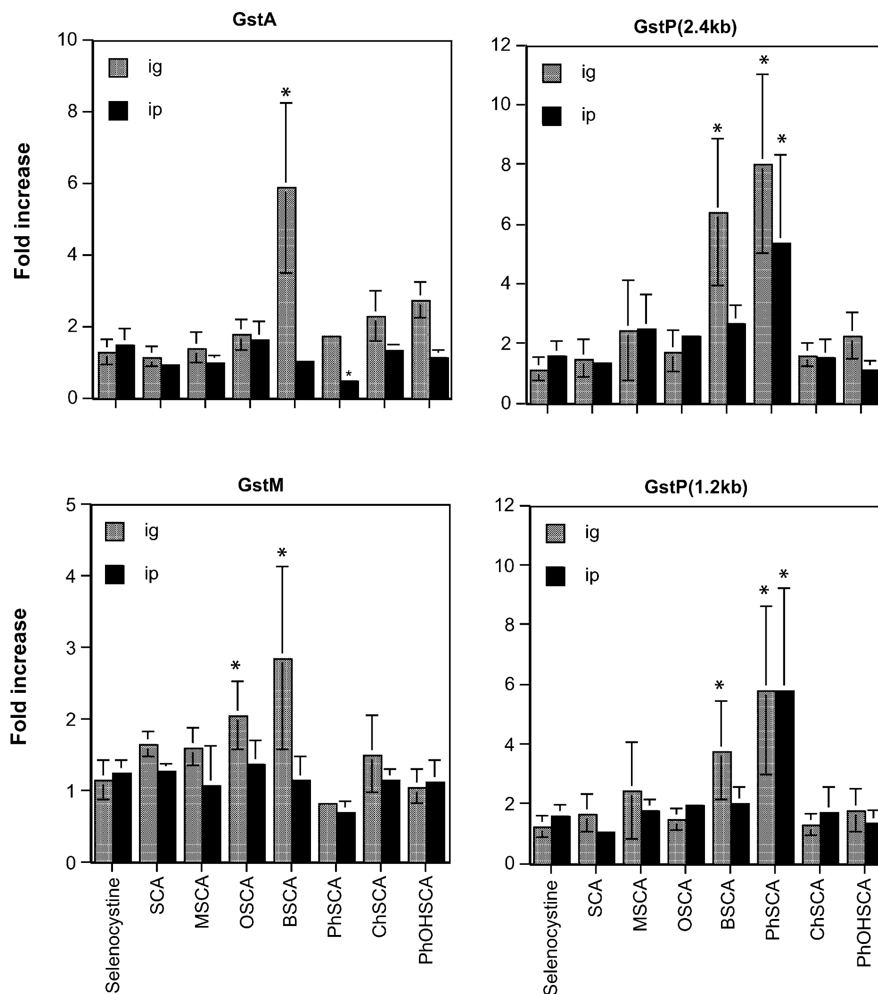

Fig. 4. Effect of selenazolidine L-selenocysteine prodrugs on mRNAs of glutathione transferases in liver. Selenazolidine compounds were administered at 1.1 mg Se/kg; L-selenocystine was administered at 0.55 mg Se/kg. SCA, MSCA, OSCA, and selenocystine were compared with the untreated animals. BSCA, PhSCA, ChSCA, and PhOHSCA were compared with the corn oil-treated animals. *Significantly different from the appropriate control, $P < 0.05$.

examining GST activities for compounds producing the greatest mRNA elevations where no increases in activity were observed (Table 4). Acute gene expression studies have the potential to yield information that can serve as an early indication of efficacy, safety and toxicity and can provide clues as to the mechanism or mechanisms involved in tumor reduction. Also, acute transcription effects more likely reflect properties of the compounds themselves, rather than secondary effects arising from their ability to maintain or enhance selenoenzymes (e.g. GPx and Tr) which though their catalytic activities on cellular constituents could also alter gene expression.

Selenium-related studies where mRNA levels have been investigated in animals have been largely confined to studies with selenoprotein mRNAs; Gpx and Tr. Early studies indicated that selenium deficiency for >40 days depressed hepatic Se-GPx mRNA levels [42,43] but the elevated levels in a selenium sufficient diet were due to cytosolic mRNA stabilization, not elevated transcription [42]. While decreases in GPx mRNA closely paralleled the degree of selenium deficiency [44] and were related to “nonsense mediated degradation” [45], supplying selenium at four-fold selenium-adequate levels did not increase levels above those seen at the selenium-adequate level [44]. Selenium deficiency also decreased Gpx1 mRNA levels in the intestines of mice [23]. In studies monitoring Tr mRNA, selenium deficiency for 28 days decreased this transcript in rat liver [46]. Selenium in the form of selenite did not alter Tr transcription [47] or Tr mRNA levels [48] in HepG2 cells but was able to stabilize Tr mRNA induced by sulforaphane. In several human cancer cell lines, selenite increased Tr mRNA

in part, or in total, by increasing mRNA stability [49]. In rats, the direction of the effects of sodium selenite (i.p. for 15 days) on hepatic Tr (and Gpx) mRNAs varied with dose, with low doses elevating, and high doses depressing [50].

In studies related to organoselenium compound effects on the mRNA levels of other chemoprotective enzymes, selenocysteine Se-conjugates induced multiple GSTA forms (2, 3, and 5) and GSTP but not GSTM in primary rat hepatocytes and H35 Reuber rat hepatoma cells [51] and *p*-XSC induced GSTs of all classes in DMBA-induced rat mammary adenocarcinomas [52]. Interestingly, selenium deficiency also induced rat hepatic GSTA mRNA [53]. In these studies, as in the present studies, elevated mRNA levels do not distinguish between elevated transcription rates and mRNA stabilization and represents a limitation to the interpretation of the possible mechanism of action of selenocompounds.

The selenazolidines that are the subject of this investigation were designed as L-selenocysteine prodrugs with different cell permeability ($C \log P$ values ranging from −3.06 to −0.51). Based on reasoning expressed earlier, the acute effect of these compounds on the transcription of a comprehensive range of chemoprotective enzymes only two of which are selenoproteins, was undertaken. Some of the selenazolidines will require enzymatic release of selenocysteine (SCA, OSCA), while the others are anticipated to release selenocysteine by spontaneous hydrolysis, albeit at likely differing rates. The variety of gene transcription responses seen could be an indication that the responses are not solely the result of the common ability of the compounds to provide cells with selenocysteine. Responses to a common entity, likely selenocysteine, may be present, as is suggested by the elevation of Nqo and Tr by four of the seven SCA compounds given i.p. (MSCA, OSCA, BSCA, and PhSCA) but responses of other genes that were not in common for these four compounds suggest that select other genes can respond to the parent compounds. However, caution is necessary in such an interpretation as there are caveats inherent in a single-dose, single-time point study. Thus, the absence of a response of a gene or genes (e.g. Nqo and Tr by SCA, ChSCA, and PhOHSCA) could be the result of inadequate generation, or inability to provide sustained concentrations of an entity such as selenocysteine from these compounds within the time frame of the investigation. These considerations also relate to the frequent differences in response to a single compound that are seen between the two routes of administration. Given i.g., the compounds are exposed on a first pass to an environment, enzymes and transporters that do not exist in the i.p. route. The mix of parent compound, metabolites or breakdown products as well as the concentrations of each to which the liver is exposed may therefore vary as a consequence.

With the common response of Nqo and Tr to many of the compounds, it is tempting to speculate that such induction might arise via the antioxidant/electrophile response element known to be present in the promoter regions of these genes [54,55], even though selenite does not act in this manner [45,47]. Evidence against is (i) that selenocystine does not elicit this induction and through the transient oxidative stress accompanying the depletion of glutathione as it is reduced to selenocysteine, might be expected to do so; (ii) GstA transcription can also be regulated by an antioxidant/electrophile response element [56–58] and was not up-regulated when both Nqo and Tr mRNAs were elevated. The frequency with which there was an absence of GstA induction when Nqo was elevated (MSCA, OSCA, BSCA and PhSCA i.p.) or vice versa (BSCA i.g.) adds substantial evidence to the idea that interaction at the ARE/EpRE is not the mechanism through which these genes are responding to the selenazolidines.

Based on the observations of the present study, and within the limitations inherent in a single-time point single-dose screen, it is apparent that instead of a series of compounds (L-selenocysteine prodrugs) with the same spectrum of properties, but differing in extent, each of the selenazolidines appears capable of activating its own spectrum of protective enzymes, or for one compound, SCA, none at all. The inducing properties appear to differ even within closely related gene families. Among the single Ugt inducers, MSCA can induce Ugt1a1, BSCA Ugt 1a6, and PhSCA Ugt 1a9. For compounds inducing two Ugts, PhOHSCA can induce Ugt1a1 and Ugt 1a6, ChSCA Ugt1a1 and Ugt1a9, and OSCA Ugt1a6 and Ugt 1a9. Similarly among the Gsts, some compounds induce none (SCA, MSCA, ChSCA, PhOHSCA), OSCA can induce GstM, PhSCA can induce GstP and BSCA can induce all three Gst classes examined, alpha, mu, and pi. For the Gst changes, the experimental approach using class-specific rather than enzyme-specific probes was designed to capture any changes, should they exist. Such an approach does have limitations in that elevations in only one member of the class may be obscured or diluted out to non-significance by other members that are not changed, or elevations in one may be cancelled out by decreases in another.

In addition to the absence of a signature mRNA response common to all the selenazolidines, there was also no pattern of response that delineated i.p. or i.g. administration of the organoselenium compounds. As indicated earlier, route of administration might be

anticipated to affect both the concentration and composition of selenocompounds (parent, hydrolysis products, and metabolites) reaching the liver and the transcriptional response may be different for each component. More extensive studies than those undertaken here would be needed to resolve the reason(s) for route differences of this single dose study, but while of possible interest for academic reasons, such differences are likely to be moot for chemoprevention studies where the compounds are likely to be included in the diet and continuously ingested. The bolus administration of the present study was viewed as a method to screen for, and accurately compare, the acute effects of a defined per animal dose of the compounds and escape any variability associated with ad libitum food ingestion.

In summary, we have demonstrated that among seven selenazolidines, all of which share the property of being L-selenocysteine prodrugs, six out of seven were able, when given as a single dose, to induce their own unique spectrum of mRNAs of chemoprotective enzymes. The seventh compound (SCA), the compound without a substitution at the 2-position, failed to significantly increase any mRNAs. We have also observed that the response observed can vary with the route by which the compound is administered, a feature likely linked to variations in the concentration, duration, and mix of selenocompounds reaching the liver. The extensive range of enzyme genes affected by selenazolidines holds promise for their possible use as chemoprotectants against toxicants with toxic effects in addition to carcinogenicity.

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