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Online Publication Date: 01 January 2009

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Oxidative Stress Due to (R)-Styrene Oxide Exposure and The Role of Antioxidants in Non-Swiss Albino (NSA) Mice

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Styrene produces lung and liver damage that may be related to oxidative stress. The purpose of this study was to investigate the toxicity of (R)-styrene oxide (R-SO), the more active enantiomeric metabolite of styrene, and the protective properties of the antioxidants glutathione (GSH), N-acetylcysteine (NAC), and 4-methoxy-L-tyrosinyl-L-glutamyl-L-cysteinyl-glycine (UPF1) against R-SO-induced toxicity in non-Swiss Albino (NSA) mice. UPF1 is a synthetic GSH analog that was shown to have 60 times the ability to scavenge reactive oxygen species (ROS) in comparison to GSH. R-SO toxicity to the lung was measured by elevations in the activity of lactate dehydrogenase (LDH), protein concentration, and number of cells in bronchoalveolar lavage fluid (BALF). Toxicity to the liver was measured by increases in serum sorbitol dehydrogenase (SDH) activity. Antioxidants were not able to decrease the adverse effects of R-SO on lung. However, NAC (200 mg/kg) ip and GSH (600 mg/kg), administered orally prior to R-SO (300 mg/kg) ip, showed significant protection against liver toxicity as measured by SDH activity. Unexpectedly, a synthetic GSH analog, UPF1 (0.8 mg/kg), administered intravenously (iv) prior to R-SO, produced a synergistic effect with regard to liver and lung toxicity. Treatment with UPF1 (0.8 mg/kg) iv every other day for 1 wk for preconditioning prior to R-SO ip did not result in any protection against liver and lung toxicity, but rather enhanced the toxicity when administered prior R-SO. The results of the present study demonstrated protection against R-SO toxicity in liver but not lung by the administration of the antioxidants NAC and GSH.

Styrene is an aromatic hydrocarbon forming a colorless liquid widely used in the manufacturing of plastics, rubber, and resins for its ability to readily form polymers. In 2006 in the United States alone, production of styrene exceeded 13 billion pounds (ATSDR, 2008). Styrene is primarily used in the manufacturing of products for everyday use, including packaging materials, insulation, fiberglass products, plastic pipes and food containers. The highest exposures occur in workers in the reinforced plastics industry. With respect to potential adverse effects of styrene on human health, a comprehensive overall review and risk assessment has been published (Cohen et al., 2002).

Styrene is both hepatotoxic and pneumotoxic in mice (Carlson, 1997; Gadberry et al., 1996). Extensive studies have shown hepatic necrosis in B6C3F1 mice administered styrene by inhalation for up to 14 d (Morgan et al., 1993a), with studies for up to 3 d demonstrating that both males and female are affected with greater mortality in male mice (Morgan et al., 1993b). These studies also showed that styrene administration produced a decrease in hepatic glutathione (GSH) levels. The studies by Gadberry et al. (1996) demonstrated that styrene and its metabolites injected ip not only produced hepatic damage in NSA mice as evidenced by increased serum sorbitol dehydrogenase (SDH) but also produced lung damage as shown by elevations in lactate dehydrogenase (LDH) and gamma-glutamyltranspeptidase (GGT) in bronchoalveolar lavage fluid (BALF). A study by Kaufmann et al. (2005) showed an increase in bronchi, bronchiolar, and alveolar cell proliferation in CD-1 mice in response to styrene oxide (3 × 100 mg/kg/d ip for 3 d). Cruzan et al. (1997) exposed Cd rats and CD-1 mice to varying levels of styrene by inhalation 5 d/wk for 13 wk and found changes in lungs of mice at exposure levels as low as 100 ppm. They also demonstrated that mice are more sensitive to styrene-induced toxicity than rats.

Styrene oxide exists in two enantiomeric forms, (R)- and (S)-styrene oxide. A difference in toxicity of the two enantiomers in mice was reported by Gadberry et al. (1996), who showed that the (R)-enantiomer exhibits a greater toxic effect than (S)-enantiomer. In three studies using the Salmonella assay, the (R)-enantiomer was found to be more genotoxic than...
the (S)-enantiomer (Pagano et al., 1982; Seiler, 1990; Sinheimer et al., 1993). Metabolism of styrene to (R)-enantiomeric styrene oxide is favored in mouse liver microsomes (Carlson et al., 1998), and metabolism is greater in mice than in rats (Hynes et al., 1999).

Styrene oxide conjugates with GSH, resulting in GSH depletion in liver and lung, which increases susceptibility to oxidative stress. Styrene exposure was shown to produce GSH depletion in liver and lung in several studies. Morgan et al. (1993b) showed a dose-dependent decrease in hepatic GSH due to styrene inhalation. Turner et al. (2005) found that styrene (600 mg/kg) and styrene oxide (300 mg/kg) ip depleted liver and lung GSH levels in mice 1 h after treatment. Significant GSH depletion was reported in rat lung and liver after a single 24-h styrene inhalation exposure at a concentration of 500 cm$^3$/m$^3$ by 66% and 16%, respectively (Elovaara et al., 1990). GSH depletion measured by a reduced number of glutathione-positive cells was also observed in bronchoalveolar epithelium of CD-1 mice after styrene oxide (3 $\times$ 100 mg/kg/d) ip injections (Kaufmann et al., 2005). Further, GSH depletion was shown in mouse lung homogenates by Gamer et al. (2004) after 21 daily styrene inhalation exposures at 160 ppm.

UPF is a synthetic tetrapeptide analog of GSH, and was synthesized in an attempt to create a synthetic reactive oxygen species (ROS) scavenger possibly for clinical use (Poder et al., 2004). Although the exact mechanism of action of UPF1 is unknown, it has been proposed to act as a direct ROS scavenger or as a signaling molecule modulating the GSH levels or the GSH/GSSG redox ratio (Karelsdon et al., 2002). The ability of UPF1 to act as a ROS scavenger was shown in studies in vivo using male Wistar rats. UPF1 (0.9 mg/kg) administration intravenously (iv) 20 min prior to induced global brain ischemia was effective in providing protection against ischemic or reperfusion injury measured by cell viability. Overall, UPF1 was shown to possess 60 times the ROS scavenging ability of GSH (Poder et al., 2004).

Styrene exposure via inhalation increases the occurrence of lung tumors in mice but not rats (Cruzan et al., 1998, 2001). Another study with pregnant O2O mice showed a statistically significant rise in the occurrence of lung tumors in comparison to male mice (Ponomarkov & Tomatis, 1978). The mechanism underlying styrene and styrene oxide toxicity leading to cancer is under investigation. Styrene 7,8-oxide has been characterized as having carcinogenic potential in several in vitro and in vivo systems as summarized by McConnell and Swenberg (1993). Styrene oxide is a reactive epoxide that readily binds to DNA, and this has been reviewed (Phillips & Farmer, 1994; Vodicka et al., 2002). However, Boogaard et al. (2000) found that formation of DNA adducts after styrene exposure was low, and it did not vary between rats and mice.

The mechanism for styrene-induced toxicity and carcinogenicity is not clear. However, styrene and its metabolites drastically reduce GSH levels in liver and lung (Morgan et al., 1993b; Turner et al., 2005; Harvilchuck & Carlson, 2006). Since this is a major antioxidant in both lung and liver, its depletion may make cells more susceptible to cellular damage due to ROS. Thus, rather than acting through a genotoxic mechanism, an explanation for styrene-induced toxicity may be oxidative stress, increasing the cellular levels of ROS and stimulating cellular replication leading to tumor formation (Harvilchuck & Carlson, 2006; Harvilchuck et al., 2008). The aim of the current study was to determine whether antioxidants could protect against styrene-induced hepatotoxicity and pneumotoxicity in mice. GSH, NAC, and UPF1 were the antioxidants compared for their abilities to prevent oxidative damage by (R)-styrene oxide. SDH activity in serum was used as a biomarker for hepatotoxicity, and LDH activity, concentration of protein, and number of nucleated cells in BALF were utilized as biomarkers for pneumotoxicity. R-styrene oxide was selected for use because of its greater toxicity than styrene or S-styrene oxide in our previous studies (Gadberry et al., 1996). Further, giving the active metabolite negates the possibility that the potential protective agents would affect the bioactivation of the styrene.

MATERIALS AND METHODS

Animals

Adult, male non-Swiss Albino (NSA) [Hsd:NSA] (25–30 g) mice were obtained from Harlan (Indianapolis, IN). The animals were housed in group cages in an AAALAC-accredited facility in environmentally controlled rooms on a 12-h light:dark cycle. The diet consisted of rodent laboratory chow (no. 5001, Purina Mills, Inc., St. Louis, MO) and tap water given ad libitum. Before being used in any experiments all animals were allowed to adapt to the animal facilities and diet for a minimum of 1 wk. Procedures and protocols were approved by the Purdue University Animal Care and Use Committee.

Chemicals

(R)-Styrene oxide was obtained from Aldrich Chemical Co. (Milwaukee, WI). Trichloroacetic acid (TCA) and fructose were purchased from Fisher (Fair Lawn, NJ). GSH, 5,5-dithiobis(2-nitrobenzoic acid) (DTNB), NADH, pyruvate, tris(hydroxymethyl)aminomethane, NaCl, KCl, NaH$_2$PO$_4$, Na$_2$HPO$_4$, and glucose were received from Sigma-Aldrich Chemical Co. (St. Louis, MO). Triethanolamine, diethyl ether, monobasic potassium phosphate, and dibasic potassium phosphate were obtained from Mallinckrodt (Paris, KY). The reagents for the bicinchoninic acid (BCA) protein assay were purchased from Pierce as a kit. All other chemicals were reagent grade or better.

Synthesis of UPF1

UPF1 was synthesized manually using Fmoc (9-fluorenymethoxycarbonyl) chemistry in a stepwise manner on Gly-Wang
resin (0.78 mmol/g). Couplings of Fmoc-protected amino acids were carried out using the standard 2-[(1H-benzotriazole-1-yl)1,1,3,3-tetramethyluronium tetrafluoroborate (TBTU) and 1-hydroxybenzotriazole (HOBT) activation in N,N-dimethylformamide (DMF). The peptides were removed from the resin and simultaneously deprotected by reaction with trifluoroacetic acid (TFA) in the presence of scavengers, water (2% v/v), 1,2-ethanedithiol (EDT, 2% v/v), and trisopropylsilane (TIS, 2.5% v/v) for 90 min at room temperature. The purity of the UPF1 peptide was >99% as demonstrated by high-performance liquid chromatography (HPLC) on an analytical Nucleosil 120-3 C18 reverse-phase column (0.4 cm × 10 cm). The molecular mass of the peptide was determined by matrix-assisted laser desorption ionization time of flight (MALDI-TOF) mass spectrometry (Voyager DE Pro, Applied Biosystems, USA), and the calculated value was obtained.

Experimental Design

Lung toxicity was investigated by measuring elevations in LDH, protein, and number of nucleated cells in BALF. Toxicity to the liver was measured by increases in serum SDH. An ip dose of 200 mg/kg NAC or an oral dose of 600 mg/kg GSH 1 h prior to 300 mg/kg R-SO ip was used to investigate whether the antioxidants prevent R-SO-induced toxicity. The dose of R-SO was selected based on previous studies (Gadberry et al., 1996; Carlson, 1997). The antioxidant doses were selected on the basis of previous studies by others (Gomez et al., 1994; Kairya et al., 2007; Terneus et al., 2007) and our preliminary experiments on the adverse effects of these agents. UPF1 (0.8 mg/kg) was given iv once 15 min prior to R-SO administration. Mice were sacrificed 12 h after R-SO administration. A repeated-dose study with UPF1 was carried out to precondition animals with UPF1, but preventing UPF1 from being available for interaction with styrene or styrene oxide in the peritoneal cavity. UPF1 (0.8 mg/kg) was administered to mice iv every other day (d 1, 3, and 5) prior to R-SO (300 mg/kg) administration ip (d 6).

Oral gavage with GSH and ip injections of NAC, UPF1, or R-SO were given as 1 ml/100 g body weight. For all studies R-SO was dissolved in corn oil. All UPF1 iv injections were given as 0.2 ml/100 g body weight.

Sorbitol Dehydrogenase (SDH) Assay

Animals were anesthetized using ethyl ether. Blood volumes of 1–1.5 ml were collected using a 22-gauge needle by cardiac puncture. Serum was collected by centrifuging the blood sample at 1160 × g for 15 min at 4°C in an Eppendorf 5810R centrifuge to precipitate cells and cellular debris. Spectrophotometric determination of serum SDH activity utilized the method of Gerlach (1983). SDH activity was measured by the decrease in absorbance of NADH at 366 nm for 2 min using a Shimadzu model UV160U ultraviolet (UV)–visible spectrophotometer. Results are expressed as micromoles per minute per liter serum.

Lactate Dehydrogenase (LDH) Assay

BALF was obtained from the anesthetized mice. The abdominal and thoracic cavities were opened. The incision was continued to the neck region, and the trachea was exposed. A small opening was made in the trachea to insert an oral feeding needle that was secured in place by thread. The lungs were perfused twice with 0.8 ml of lavage fluid for a total volume of 1.6 ml. The fluid consisted of NaCl (145 mM), KCl (5 mM), NaH₂PO₄ (1.9 mM), Na₂HPO₄ (9.4 mM), and glucose (5.5 mM) at pH 7.4. LDH activity was measured by the spectrophotometric method of Vassault (1983) in centrifuged BALF samples. BALF fluid (0.1 ml), NADH (0.24 mM), and Tris (81 mM)/NaCl (203 mM) buffer (pH 7.2) were incubated at 30°C for 15 min. The reaction was initiated by the addition of 0.5 ml pyruvate (9.8 mM) to make a total volume of 3 ml. The activity of LDH was measured 30 s after the addition of pyruvate by the decrease in absorbance of NADH at 339 nm for 2 min. Results are expressed as micromoles per minute per liter BALF.

Bicinchoninic Acid (BCA) Protein Assay

The BALF was centrifuged at low speed, 1100 × g, to remove cellular debris. The amount of protein was determined in BALF supernatant with the bicinchoninic acid method by following the directions of the Pierce BCA kit. The amount of BALF used in the assay was diluted 50 μl to 50 μl of water. Two milliliters of 50:1 AB (A = containing sodium carbonate, sodium bicarbonate, bicinchoninic acid and sodium tartrate in 0.1 M sodium hydroxide; B = 4% cupric sulfate) solution were added to the 100 μl of sample–water mixture. Bovine serum albumin (BSA) standards were prepared fresh from 2 mg/ml BSA stock. Test tubes were incubated 30 min at 37°C. Absorbance of each sample was measured at 562 nm. The results are expressed in micrograms protein per milliliter BALF.

Cell Counts in BALF

The number of cells in 100 μl BALF was counted using a hemocytometer. Equal amounts of trypan blue and BALF were mixed, and all the nucleated cells were counted. Results are expressed as cells per microliter BALF.

Statistical Analysis

Values are means ± standard error of mean. Three to eight animals per group were used in this study. Data analysis was carried out by using analysis of variance (ANOVA) followed by Tukey’s test for significance at p < .05. The computer program used for analysis was KaleidaGraph version 3.6. Due to heterogeneity of the data, in some studies logs of
RESULTS

NSA mice, as originally used in the studies by Gadberry et al. (1996), were utilized due to their greater sensitivity to styrene and styrene oxide-induced liver and lung toxicity than some other strains (Carlson, 1997). R-SO produced increased activities in SDH and LDH (Figure 1). Four of 10 mice from the saline + R-SO treatment group died before 12 h and were excluded from the study. GSH oral administration protected against hepatotoxicity, decreasing the SDH activity in GSH + R-SO treated mice in comparison to saline + R-SO treated mice. LDH activity was significantly increased in both R-SO treated groups in comparison to saline control (Figure 1). These results indicate that GSH administration was not able to protect against R-SO toxicity in lung. Protein analysis of BALF showed no significant differences in levels following any treatment (Figure 1). The saline + R-SO treated group had significantly increased cell counts in comparison to GSH + corn oil treated group (Figure 1). The value for the GSH + R-SO group was in between the two control groups and the saline + R-SO group.

A similar study was carried out to investigate whether NAC protects against liver and lung toxicity. NAC (200 mg/kg) ip was administered 1 h prior to R-SO exposure. Mice were sacrificed after 12 h. Two of 9 mice from the 300 mg/kg NAC + R-SO treatment group and 1 from the saline + R-SO treatment group died before the 12-h endpoint and were excluded from the study. SDH activity in the saline + R-SO treated group was significantly increased in comparison to other treatments (Figure 2). These data indicate protection against liver damage by NAC. LDH activity was significantly increased in both the saline + R-SO and NAC + R-SO treated groups in comparison to the saline and NAC controls (Figure 2) indicating a lack of protection in the lung. Protein levels in BALF were not, however, significantly different among treatments (Figure 2), whereas cell counts were significantly increased in saline + R-SO treated group in comparison to saline + corn oil control indicating

FIG. 1. Effect of oral administration of GSH on hepatotoxicity and pneumotoxicity induced by R-SO in NSA mice. NSA mice were treated with an oral dose of GSH (600 mg/kg) 1 h prior to (R)-styrene oxide (R-SO) (300 mg/kg) ip injection. Saline was used as a control for GSH administration and corn oil as a control for styrene administration. Animals were euthanized 12 h after styrene administration. Four of 10 mice from the saline + R-SO treatment group died before 12 h and were excluded from the study. SDH and LDH activities are expressed as micromoles per minute per liter. Protein concentration in BALF is expressed as micrograms protein per milliliter BALF. Number of cells in BALF is expressed as cells per microliter BALF. Bars with different superscript letters are significantly different from one another at $p < .05$ (means ± SEM, $n = 5–8$).
significant pneumotoxicity by R-SO (Figure 2). The NAC + R-SO group value was intermediate.

UPF1 was used in these studies for its ROS scavenging ability. In order to test the role of UPF1 in protecting against liver and lung toxicity, an iv dose (0.8 mg/kg) was given 15 min prior to R-SO administration (300 mg/kg) ip. There was a significant difference in SDH activity in mice treated with UPF1 + R-SO in comparison to all other treatment groups, indicating that UPF1 produced a synergistic effect when combined with R-SO (Figure 3). LDH activity was substantially higher in the UPF1 + R-SO treated group than in other treatment groups (Figure 3). R-SO (300 mg/kg) alone did not produce significant lung toxicity in mice, but when given with UPF1 it produced a significant increase in toxicity in comparison to the saline + corn oil control group. The amount of protein in BALF was not significantly different among treatment groups except the controls. The number of cells present in BALF was significantly different in the saline + R-SO and UPF1 + R-SO treated groups in comparison to saline and UPF1 controls (Figure 3).

A 1-wk study with UPF1 was carried out to investigate if preconditioning with UPF1 might make the molecule more readily available and thus effective in preventing liver and lung damage induced by R-SO. UPF1 (0.8 mg/kg) was administered to mice iv every other day for 3 d (d 1, 3, and 5) prior to R-SO (300 mg/kg) exposure ip (d 6). Animals were sacrificed 12 h after the styrene administration (d 7). Two of 4 mice from the saline + R-SO treatment group died before the 1-wk endpoint and were excluded from the study. There were no significant differences in SDH activity among different treatments, although the saline + R-SO and UPF1 + R-SO treated groups had elevated activities (Figure 4). LDH activity was significantly increased in the UPF1 + R-SO treated group in comparison to saline + corn oil control (Figure 4). Protein concentration did not vary with treatment (Figure 4). Cell counts in the

![SDH activity](image1)

![LDH activity](image2)

![Protein concentration](image3)

![Cells in BALF](image4)

**FIG. 2.** Effect of ip administration of NAC on hepatotoxicity and pneumotoxicity induced by R-SO in NSA mice. NAC (200 mg/kg) was administered ip 1 h prior to R-SO (300 mg/kg). Controls were administered corn oil. NSA mice were sacrificed 12 h after R-SO administration. Two of 9 mice from the NAC + R-SO treatment group and 1 from the saline + R-SO treatment group died before 12 h and were excluded from the study. SDH and LDH activities are expressed as micromoles per minute per liter. Protein concentration in BALF is expressed as micrograms protein per milliliter BALF. Number of cells in BALF is expressed as cells per microliter BALF. The logs of the SDH, LDH, and protein data were used for statistical comparison. Bars with the same superscript letters are not significantly different from one another at \( p < .05 \) (means ± SEM, \( n = 4–9 \)).
saline + R-SO and UPF1 + R-SO treated groups were significantly higher in comparison to saline and UPF1 controls (Figure 4). This indicates significant lung damage by R-SO, yet shows no protection by UPF1.

DISCUSSION
Styrene is metabolized to styrene oxide by cytochromes P-450 in the liver and lung, where the adverse effects are seen as a result of oxidative stress. GSH forms a conjugation product with styrene oxide, increasing the solubility of this toxic metabolite and enhancing its excretion from the body, which at the same time leads to depletion of GSH. This pathway is much more prevalent in rodents than in humans (Sumner & Fennel, 1994; Johanson et al., 2000; Vodicka et al., 2006). Styrene oxide exists in two enantiomeric forms, (R)- and (S)-styrene oxide. The difference in toxicity of the two enantiomers in mice was reported by Gadberry et al. (1996). The (R)-enantiomer exhibits a more toxic effect than (S)-enantiomer. Because of the more potent toxicity of R-SO, it was used in the current studies. GSH is essential for preventing oxidative stress and thus cellular damage. This study was based on previous experiments and success by other researchers who utilized GSH, GSH precursor NAC, or GSH analogs as a means of maintaining cellular GSH levels or preventing tissue damage induced by oxidizing agents. Terneus et al. (2007) showed that ip administration of NAC (1.25 mmol/kg) immediately prior to acetaminophen (300 mg/kg) maintained the hepatic GSH levels and protected against centrilobular liver necrosis. Another study showed that NAC (2.5 mmol/kg) ip 1 h prior to acetaminophen (5 mmol/kg) treatment was effective in preventing increases in plasma alanine aminotransferase (ALT) activities (Gomez et al., 1994). Kariya et al. (2007) found that 1 h after an oral dose of 300 mg/kg GSH, GSH levels in lung tissue increased twofold. A study by Vina et al. (1989) showed that administration of GSH orally was sufficient to increase GSH levels in fasted rats, as well as in mice treated with the GSH-depleting agent diethyl maleate.

The current study was carried out to evaluate the protective role of GSH in R-SO induced toxicity by administering saline...
or GSH (600 mg/kg) orally 1 h prior to dosing with either corn oil or R-SO (300 mg/kg) dissolved in corn oil. After 12 h there was a significant difference between saline + R-SO treated and all other treatment groups in SDH activity (Figure 1). This indicates that the mice exhibited hepatotoxicity in response to R-SO, and administration of GSH prevented the damage.

Pneumotoxicity measured by LDH activity in BALF showed a different outcome, indicating that R-SO (300 mg/kg) produced a significant increase in LDH activity and number of cells in BALF, yet this was not prevented by administration of GSH (Figure 1).

NAC was administered ip 1 h prior to R-SO (300 mg/kg) administration. Although there was heterogeneity within the R-SO treated group, the results showed increased SDH activity (Figure 2). From these data it can be concluded that NAC treatment did prevent liver damage. Similarly, LDH activity and the number of cells in BALF were significantly increased in saline + R-SO and NAC + R-SO groups, indicating that R-SO did produce significant pulmonary toxicity at the given dose. However, NAC did not prevent lung damage. Protein analysis of BALF did not show any differences between treatment groups.

As shown by Poder et al. (2004), UPF1 is a potent antioxidant GSH analog, with the capability to act 60 times more efficiently as an ROS scavenger in comparison to GSH. In view of this, the role of UPF1 in preventing hepatotoxicity and pneumotoxicity induced by R-SO was investigated. The reason for the lack of protection by UPF1 administered iv 15 min prior to R-SO (300 mg/kg) ip could be related to dispositional problems prior to its reaching and being taken up by the target tissues. From these results it can be concluded that UPF1 administration alone was not responsible for producing hepatotoxicity or pneumotoxicity measurable by SDH, LDH, or BALF protein assays and cell counts (Figure 3). However, the UPF1 + R-SO treated group showed a synergistic effect in liver and lung toxicity measured by SDH and LDH activity. Why
this occurred is unknown. These results may imply the existence of an additional mechanism not yet reported, by which UPF1 increases styrene induced liver and lung toxicity in the mice or UPF1 reacts directly with S-RO to form a more toxic product. The mortality rate was also high in the UPF1 + R-SO treatment group in comparison to others, making it likely that the remaining mice were not representative of the whole treatment group, since sample collection was not possible from the dead mice. Thus, the value in this group may be underestimated. A 1-wk preconditioning experiment with UPF1 (0.8 mg/kg) iv resulted in no significant protection against liver and lung toxicity in the mice (Figure 4).

In summary, the conclusion drawn from the present studies is that in NSA mice both NAC and GSH have the potential to significantly protect against R-SO-induced liver toxicity. This effect was not, however, seen against lung toxicity. The reason for the difference between the tissues is not clear since it was readily apparent that R-SO did cause pneumotoxicity under the conditions of this study. It may be related to the uptake of the protective agents by the liver but not the lung. A final conclusion was that the synthetic GSH analog UPF1 was not proficient in preventing liver and lung tissue damage produced by R-SO exposure, but rather it resulted in a more toxic combination when administered in combination with R-SO. Future studies may consist of investigating different antioxidants, such as vitamin C or GSH analogs, and their ability to protect against liver and lung damage.

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