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PROTECTION BY CYSTEINE ESTERS AGAINST CHEMICALLY INDUCED PULMONARY OEDEMA

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Abstract—Perfluoroisobutene (PFIB) is a hydrophobic reactive gas produced by the pyrolysis of polytetrafluoroethane which induces pulmonary oedema similar to that induced by phosgene when inhaled. When a lethal dose is inhaled by Porton strain rats total non-protein thiol (NPSH) and glutathione (GSH) in the lung are reduced by between 30 and 49%, respectively. If the endogenous levels of thiols in the lung are reduced by pretreatment with buthionine sulfoximine (BSO) 16 hr before exposure to PFIB, the rats become more susceptible to the effects of the gas. The effect of BSO pretreatment on toxicity was prevented by pretreatment 30 min before exposure, with 5 mmol/kg *N*-acetylcysteine (NAc). NAc increased the levels of cysteine (CySH) in the lung by 150% and GSH was unaffected. Similarly pretreatment with 3 mmol/kg CySH also protected against toxicity and raised CySH levels by 100%. A series of cysteine esters and cystine dimethyl ester (CDME) have been synthesised which selectively raise lung levels of CySH in the rat lungs after intraperitoneal (i.p.) injection. The methyl ester and CDME raised lung levels of CySH by 4000 and 2000%, respectively, 10 min after i.p. injection whilst GSH levels remained unchanged. Cysteine isopropyl ester raised lung levels of CySH by 10,600% but liver levels by only 1400%. All esters except the *t*-butyl ester (CTBE) also raised maximal plasma levels of NPSH by up to 500%; however, when NAc was injected plasma levels increased by over 1500%. Rats treated with these esters at 3 mmol/kg and with NAc at 5 mmol/kg were protected against lethal doses of PFIB in all cases except when CTBE was used. It appears that these cysteine esters may distribute preferentially into the lung, unlike NAc. The selective enhancement of pulmonary CySH levels may provide a method for the protection of lungs against inhaled reactive toxicants by increasing intracellular CySH. Levels of CySH may also be raised in epithelial lining fluid thus reducing access of gaseous toxicants to pulmonary tissue.

Chemically induced pulmonary oedema produced by the inhalation of smoke from fires, the pyrolysis products of plastics and gases such as phosgene is difficult to treat clinically [1, 2]. The nature of the pulmonary damage produced varies with the composition and chemical nature of the gas or smoke but includes damage to the airways and alveoli leading to oedema which may only be manifest after a latent period [3, 4]. Corticosteroids are administered frequently to victims but the evidence for beneficial effects is limited [1, 2].

Many gaseous pulmonary toxicants are highly reactive electrophiles and the interaction at the cellular level within the lung is a function of their relative hardness or softness as electrophiles and their solubility or partition coefficients. Perfluoroisobutene (PFIB†), a pyrolysis product of polytetrafluoroethene, is a highly hydrophobic gas with an LCt_{50} of 1250 mg min/m^3 (concentration of 125 mg/m^3 for 10 min) to rats that causes little damage to the upper airways; most cellular damage is in the lower respiratory tract involving the capillary endothelium, Type I and II pneumocytes [5]. Compounds such as this produce similar pulmonary effects to those of phosgene although reported toxicity is greater [6–10].

This study sought to determine in the lungs the effect on the cellular thiol nucleophilic protectants, GSH and CySH of exposure to PFIB, and to identify compounds that might increase the levels of protectant in the lungs to prevent the effects of inhaled toxicants. A series of cysteine esters has been identified which selectively increases the levels of lung cysteine and will prevent the death of rats exposed to supra-lethal doses of PFIB. The mechanism of this protection has been investigated.

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† Abbreviations: PFIB, perfluoroisobutene; NPSH, non-protein thiol; GSH, glutathione; CySH, cysteine; BSO, buthionine sulfoximine; NAc, *N*-acetyl CySH; CME, CySH methyl ester; CDME, cystine dimethyl ester; CIPE, CySH iso-propyl ester; CNBE, CySH *n*-butyl ester; CIBE, CySH iso-butyl ester; CTBE, CySH *t*-butyl ester; CNPE, CySH *n*-pentyl ester; CCHE, CySH cyclohexyl ester; mBBr, monobromobimane; i.p., intraperitoneally; PE, Perkin–Elmer; Ct, concentration with time; LCt_{50}, median lethal concentration with time.

MATERIALS AND METHODS

Animals. Female Porton strain rats weighing 170–200 g were used throughout this study.

Chemicals. DL-BSO, NAc, L-CySH, L-CME, and L-CDME were obtained from the Sigma Chemical Co. (Poole, U.K.). CIPE, CNBE, CIBE CTBE, CNPE and CCHE mBBr and PFIB (purity >96.1%) were synthesized at CDE. All esters were synthesized by a method analogous to that described below for CIPE.

Preparation of CIPE hydrochloride. Anhydrous

hydrogen chloride (25 g) was passed into propan-2-ol (228 mL). L-CySH (15 g) was added and the mixture was heated under reflux for 4 hr. The solution was concentrated to half its original volume and the crystalline product was filtered off, and recrystallized from propan-2-ol to yield CIPE hydrochloride (19.5 g, 81%) m.p. 155–156°. Identity and purity were confirmed by HPLC and NMR, and i.r. spectroscopy.

L-CNBE hydrochloride (76%, m.p. 84°), L-CIBE hydrochloride (83%, m.p. 87°), L-CNPE hydrochloride (55%, m.p. 87°) and L-CCHE hydrochloride (75%, m.p. 130°) were prepared similarly. L-CTBE hydrochloride (78%, m.p. 213–214°) was prepared by a similar method but anhydrous HCl was passed into the refluxing mixture for 5 days.

Dose regimes. DL-BSO and NAc were dissolved in phosphate-buffered saline (pH 7), and administered at 4 mmol/kg and 3, or 5 mmol/kg, respectively. CySH and its esters were dissolved in physiological saline and administered at 1.5, or 3 mmol/kg. All injections were performed i.p.

Lung perfusion. Before the measurement of lung thiols all experimental animals were anaesthetized with 5 mL/kg (60 mg/mL) of Sagatal (May and Baker, Dagenham, U.K.) i.p. When the animal was fully anaesthetized, the trachea was exposed and cannulated. The abdominal and thoracic cavities were then opened and the posterior vena cava was cut to exsanguinate the animal. An incision was then made in the left atrium and into the right ventricle. A cannula fitted with a supply of heparinized 0.9% saline, at a flow rate of 20 mL/min, was then passed through the right ventricle into the pulmonary artery. Saline was allowed to flow through the lungs whilst they were gently inflated and deflated manually using a syringe fitted to the tracheal cannula. The lungs became pale in colour shortly after perfusion commenced but the perfusion was continued until the perfusate emerged colourless (approx. 45 sec). The lungs were then removed, washed in physiological saline and the wet weight recorded.

Measurement of the total NPSH content of the lung and plasma. Measurement of all thiols in this study was carried out at the same time each day (10.00–11.30 a.m.) and animals were always exposed to PFIB at the same time each day (09.30 a.m.). The total NPSH in the lung and plasma was measured using a modified Ellman assay [11]. Lung: approximately 200 mg portions from the right lobe were placed immediately in 2 mL of 4% (w/v) sulphosalicylic acid (BDH, Poole, U.K.), homogenized in a Potter–Elvehjem, Teflon–glass homogenizer and centrifuged at 3000 *g* for 10 min at 4°. Supernatant (0.5 mL) was removed and added to 4.5 mL of DTNB reagent (0.1 mM 5,5′-dithiobis-2-nitrobenzoic acid in phosphate buffer pH 8.0). Plasma: 0.1 mL of plasma was added to 4.5 mL of DTNB reagent. Colour was allowed to develop for 10 min and the absorbance determined at 412 nm on a Pye Unicam 1800 spectrophotometer. The calibration curve was prepared from GSH.

Measurement of GSH and CySH. Reduced GSH and CySH were measured by HPLC using a modification of the method of Kosower *et al.* [12], and Fahey and Newton [13] based upon the reaction of mBBr with sulphydryl groups to form highly fluorescent adducts.

Approximately 30 mg portions of lung prepared as described above (at the same time and from the same lung lobe) were added to 0.9 mL of mBBr solution (0.5 mg/mL in 0.4% acetonitrile, 99.6% 0.05 M *N*-ethylmorpholine pH 8.0). Samples were homogenized using a Potter–Elvehjem homogenizer for 15 sec and then incubated at 25° for 1 hr. When incubation was complete 0.1 mL of methane sulphonic acid (10% v/v) was added to precipitate protein and the mixture re-homogenized. Samples were centrifuged at full speed in an MSE Micro-Centaur centrifuge for 4 min and supernatants removed for HPLC analysis. Samples were assayed immediately or stored at −20° until required.

HPLC analysis for mBBr derivatives of thiols was carried out using one of the following systems: (a) Samples were injected via a Rheodyne 20 μL loop injector onto a Hypersil ODS 3 micron HPLC column. The column was 10 cm long by 4.9 mm i.d. fitted with a C_{18} Corasil guard column. This was coupled to a Waters 6000A HPLC pump operating at approximately 2000 psi. Isocratic elution was carried out with a mobile phase of 8% acetonitrile, 91.75% HPLC water (Hipersolv, BDH) and 0.25% glacial acetic acid adjusted to pH 3.7 with a small volume of concentrated NaOH. This was followed by a flushing solvent of 100% acetonitrile to remove other residual thiol derivatives which were not assayed. The eluent was detected in a PE LS-5 spectrophotofluorimeter fitted with a 25 μL flow cell accessory and coupled to a Hewlett–Packard Model 3393A integrator. (b) Samples were injected via a PE ISS200 autosampler onto a 15 cm × 4.6 mm, 3 micron ODS C_{18} column fitted with a Corasil guard column. This was coupled to a PE LC250 binary HPLC pump operating at approximately 2600 psi. Elution was stepped using 91% of mixture A (0.25% glacial acetic acid, 99.75% HPLC water; Rathburn Ltd) adjusted to pH 3.7 with NaOH) and 9% acetonitrile for 5 min followed by 60% of mixture A, and 40% acetonitrile for 10 min. A 10 min re-equilibration with the initial eluant was allowed before injection of subsequent samples. Fluorescent derivatives were detected on a PE LS-4 spectrophotofluorimeter coupled to an Amstrad PC1640 operating PE software package 2100.

Measurement of DNA content. The method of Cesarone *et al.* [14] was used. From a homogenate of tissue (1/8 w/v) in physiological saline buffered with phosphate/saline buffer at pH 7.4, 30 μL was added to 4.97 mL of phosphate/saline buffer (pH 7.4) and 1 mL of the dye, bis-benzamide (Hoechst No 33258) (6 μg/mL). The sample was mixed and the fluorescence determined in a PE LS-5 spectrophotofluorimeter after 2 min (excitation wavelength 352 nm, slit width 2.5; emission wavelength 456 nm, slit width 5).

Exposure to PFIB. Exposures were carried out in the Toxicology Section, Biology Division, CBDE. Measured quantities of gas were released from a storage cylinder via a needle valve and were mixed with nitrogen in Lamofoil bags. The gas mixture was passed into an air stream of known flow and the

Table 1. Decrease in pulmonary NPSH and GSH after exposure to toxic dosages of PFIB

Group	Dosage (mg min/m³)	NPSH $2(μmol/mg DNA ± SD)	GSH
Control	—	0.80 ± 0.27	0.50 ± 0.28
LCt_{50}	1250	0.50 ± 0.17†	0.26 ± 0.09‡
Control	—	0.85 ± 0.20	0.50 ± 0.13
LCt_{100}	2000	0.52 ± 0.10*	0.36 ± 0.11‡

The lungs were removed from matched groups of nine rats between 15 and 45 min after the commencement of a 10-min exposure to PFIB. NPSH and GSH were determined as described in Materials and Methods. *, † and ‡ indicate that results were significantly different at the $P < 0.001$, 0.01 and 0.05 levels, respectively.

Table 2. Protection by thiols against the lethal effects of PFIB in rats pretreated with BSO

Pretreatment	PFIB (mg min/m³)	Survivors Control	Treated
BSO/NAc	1653	0/5	5/5
BSO/CySH	1653	0/5	4/5
BSO/CME	1669	0/5	3/5
BSO/CIPE	1669	0/5	2/5
BSO/CDME	1637	0/5	2/5

Groups of five rats were injected i.p. with 4 mmol/kg BSO 16 hr and 3 mmol/kg CME or CIPE, 5 mmol/kg NAc, or 1.5 mmol/kg CDME 30 min before exposure to PFIB. Results are expressed as: number of rats that survived to 7 days/number of rats exposed. Control rats were not injected with either BSO or thiol.

mixture of gas and air was then drawn through the exposure chamber which contained the rats. Gas concentrations were monitored, prior to passing through the exposure chamber, by passage through a Miran Model 1A i.r. analyser which had been calibrated previously with known concentrations of gas. All exposures were 10 min in duration. The gas is non-irritant and animals showed no behavioural anomalies during exposure.

Protection studies. To assess the protection by thiols against exposure to PFIB, groups of five or six rats were injected i.p. with thiol, and 30 min later exposed as described above. After exposure they were kept for 7 days in a quiet environment with a 12 hr controlled light/dark cycle. Preliminary studies had shown that a noisy and stressful environment exacerbated the severity of the response to PFIB.

RESULTS

Levels of thiols in tissues in this study have been related to DNA levels in the tissues to avoid distortions that might be introduced by the influx of oedema fluid into the lungs of rats exposed to PFIB. In normal Porton strain rats the amount of DNA/g wet wt of tissue was found to be 4.86 ± 0.306 (± SE) for lung and 5.57 ± 0.19 (± SE) for liver (N = 28). In rats exposed to Cts of 1250 mg min/m³ or 2000 mg min/m³ of PFIB the levels of NPSH and GSH were significantly less (30–49%) than in untreated animals (Table 1). There was no significant difference in the extent of thiol reduction between the two exposure dosages.

To determine whether the depletion of cellular GSH was a significant factor in the toxicity of PFIB rats were treated with BSO prior to exposure. Preliminary experiments with groups of animals that had been pretreated with BSO showed that levels of NPSH, including GSH, in lung were not reduced to their maximum extent until between 10–18 hr after dosing with BSO. Consequently, measurement of lung thiols and exposure to PFIB were carried out 16 hr after treatment with BSO. At this time BSO significantly decreased lung NPSH and GSH levels by 38.9 and 56.5%, respectively. Rats exposed to PFIB only did not show signs of increased respiratory rate until 4–6 hr after exposure and five out of six had died by 22 hr. Rats which had been pretreated with BSO were, however, much more susceptible to the toxic effects of PFIB: four out of six of the rats had died by 7.5 hr after exposure and all the rats showed signs of respiratory distress sooner than those in the control group.

Another group of rats pretreated with BSO, as described above, were injected with 5 mmol/kg NAc, 30 min before exposure. When these rats were exposed to PFIB (Ct 1653 mg min/m³) they were totally protected against the lethal effects of PFIB. CySH, CME, CIPE (3 mmol/kg) and CDME (1.5 mmol/kg) were also administered 30 min before exposure to rats pretreated with BSO, and these rats were also protected to some degree against the lethal effects of PFIB (Table 2).

The ability of NAc, CySH and its esters to protect against the lethal effects of PFIB alone were determined by administration of the thiol 30 min before exposure to PFIB (Table 3). NAc provided complete protection when administered at 5 mmol/kg. However, this dose was not well tolerated and rats showed signs of discomfort, and gastro-intestinal tract disturbance after administration. NAc at 3 mmol/kg was better tolerated but it was not as effective a protectant; only three out of five rats survived compared to five out of five in the higher dose group. CySH and all its esters, with the exception of CTBE, dosed at 3 mmol/kg gave a high degree of protection in all cases against a supralethal dose of PFIB. Rats appeared to be normal with regard to their activity and behaviour at all times after ester and PFIB administration. CDME at 3 mmol/kg was not well tolerated by rats but at 1.5 mmol/kg signs of discomfort were absent and all rats were protected against a supra-lethal dose of PFIB.

To determine the fate of CySH, CME (3 mmol/kg), CDME (1.5 mmol/kg) and NAc (5 mmol/kg) *in vivo*, groups of three rats were injected i.p. with each compound and killed at selected time points after thiol administration. Blood samples were taken by cardiac puncture and after perfusion thiol levels

Table 3. Protection of rats by thiols when exposed to lethal dosages of PFIB

Pretreatment thiol	Dosage (mmol/kg)	PFIB (mg min/m³)	Survivors PFIB alone	Survivors PFIB+ thiol
CySH	3	1625	0/6	6/6
NAc	3	1596	0/5	3/5
NAc	5	1669	0/5	5/5
Esters				
CME	3	1612	0/6	5/6
CIPE	3	1669	0/5	5/5
CNBE	3	1650	0/5	5/5
CIBE	3	1650	0/5	5/5
CTBE	3	1650	0/5	0/5
CNPE	3	1650	0/5	5/5
CCHE	3	1650	0/5	5/5
CDME	1.5	1625	0/6	6/6

Rats were injected i.p. with thiol 30 min before exposure to PFIB. Results are expressed as: number of rats that survived for 7 days/number of rats exposed.

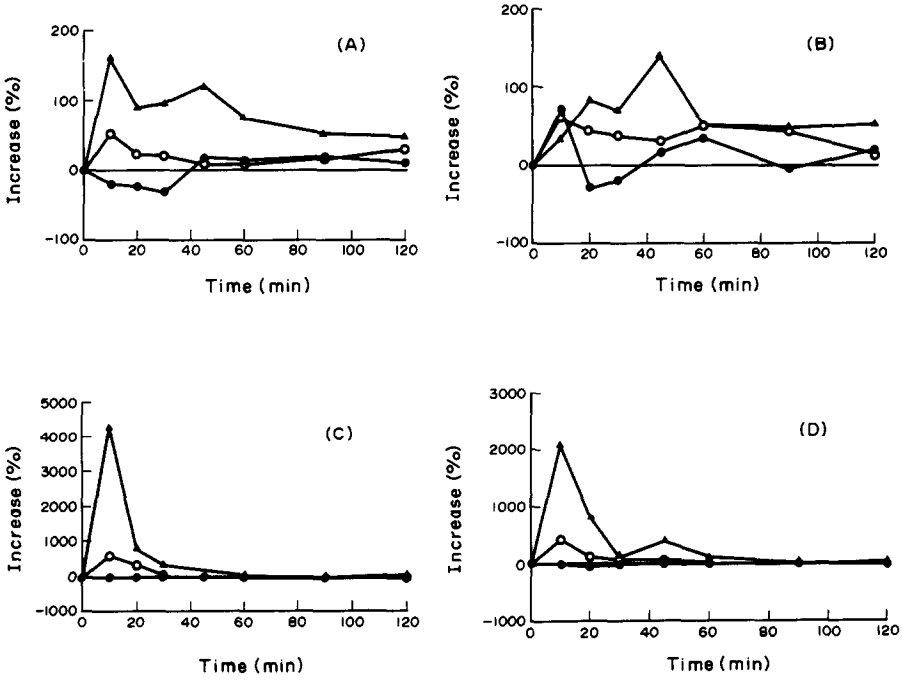

Fig. 1. Mean % increase in NPSH, GSH and CySH levels in the lungs of rats injected i.p. with either (A) CySH (3 mmol/kg), (B) NAc (5 mmol/kg), (C) CME (3 mmol/kg) or (D) CDME (1.5 mmol/kg). (○) NPSH; (●) GSH; (▲) CySH. Results are the means of data from three rats killed at each time point.

in the lung, and plasma were determined (Fig. 1A–D). CySH and NAc increased lung levels of CySH by just over 100 and 150% at 10 and 40 min, respectively. CME and CDME, however, increased lung levels of CySH by over 4000 and 2000%, respectively, 10 min after injection. In all cases the levels of GSH within the lung remained unchanged or decreased slightly. Concomitant with these changes the plasma levels of NPSH were maximal at 10–20 min with the greatest elevation of NPSH produced by NAc (5 mmol/kg) between 10 and 30 min after injection (Fig. 2).

Further investigation of the degree of elevation of plasma NPSH levels was undertaken in order to identify cysteine esters which might give prolonged protection against inhaled toxicant. In this study, blood samples were taken from groups of three rats anaesthetized and cannulated in the carotid artery. After a control sample of blood was taken the rat was injected i.p. with cysteine ester at 3 mmol/kg.

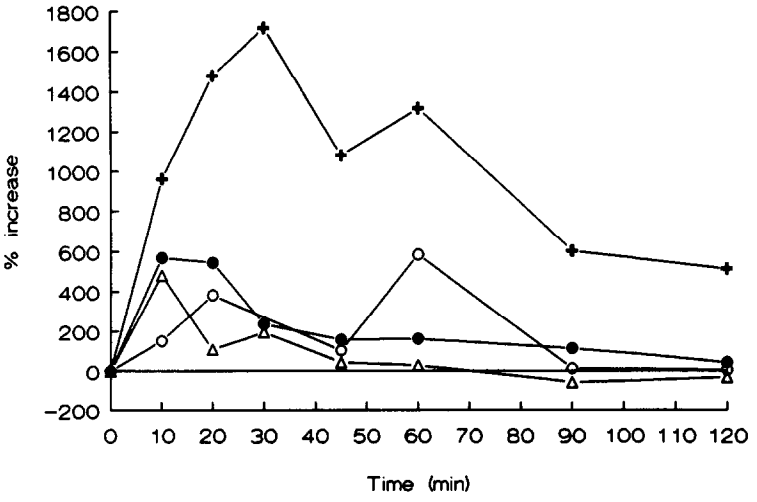

Fig. 2. Mean % increase in NPSH in the plasma of rats injected i.p. with either CySH (3 mmol/kg) (●); NAc (5 mmol/kg) (+); CME (3 mmol/kg) (△) or CDME (1.5 mmol/kg) (○). Results are the means of data from the same three rats as in Fig. 1, killed at each time point.

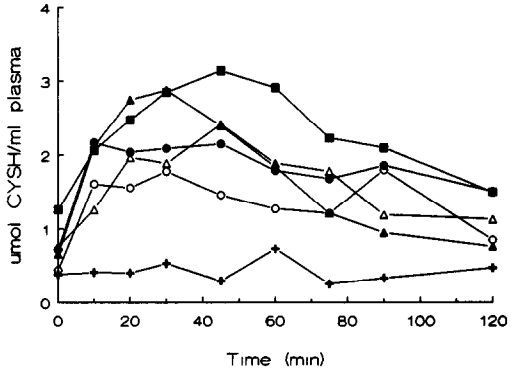

Fig. 3. Mean levels of NPSH in the plasma of rats injected i.p. with 3 mmol/kg of either CIPE (▲); CNBE (△); CIBE (■); CTBE (+); CNPE (○) or CCHE (●). Results are the means of data from three rats cannulated in the carotid artery and sampled at time points during the 120-min experiment.

The mean plasma levels of NPSH are presented in Fig. 3. CIPE and CIBE produced the greatest increase in plasma NPSH levels, and levels were sustained the longest with CIBE, CCHE, CNBE and CIPE in decreasing order. CTBE did not raise plasma levels of NPSH above control levels during the 120 min experiment.

CIPE was selected for further investigation and the levels of NPSH, GSH and CySH were determined in the lungs, liver and plasma of groups of four rats at selected time points after i.p. injection of CIPE at 3 mmol/kg (Fig. 4A–C). CIPE elevated lung levels of CySH from baseline values of 0.06 μmol/mg DNA to 6.42 μmol/mg DNA—an increase of over 10,000%—between 17 and 20 min after the administration of CIPE (Fig. 4A). Lung levels of GSH remained unchanged. In the same rats, liver levels of CySH (Fig. 4B) increased but to a much lesser extent from 0.11 μmol/mg DNA to 1.62 μmol/mg DNA (1400%) at the earlier time of 10–12 min. Levels of GSH in the liver although higher than those in the lung were similarly unchanged after CIPE administration. CIPE was not detected in either the lung or the liver. Levels of CySH had returned to normal by 90 min in the lung and 45–60 min in the liver. In the plasma from these rats (Fig. 4C) peak levels of NPSH were seen at 10 min after injection with a steady decrease to control levels by 2 hr.

DISCUSSION

The pattern of pulmonary damage induced by PFIB is characteristic of highly hydrophobic gases which penetrate into the deep lung; gases which are predominantly water soluble elicit most of their effects in the upper respiratory tract [15]. Chronologically the first sign of cellular damage is at the pulmonary epithelium; however, there is evidence of interstitial oedema and increased lymph flow before alveolar filling suggesting that damage to endothelial cells is an early event (Brown *et al.*, personal communication). After exposure to an LCt_{50} there is no increase in lung wet weight for up to 6 hr, after which there is a progressive filling of the alveoli which may lead to death from pulmonary oedema between 8 and 12 hr after an LCt_{50} dosage. No pathology is apparent outside the lung apart from congestion induced by flow resistance resulting from severe pulmonary oedema.

Thiols have several major roles within cells which include the function of GSH as a cofactor in transport and transferase reactions, protein synthesis, and in the detoxification of reactive intermediates formed intracellularly [16]. Endogenous levels of CySH are low although its reported toxicity relates mainly to neonatal animals with incomplete blood–brain barriers [17]. Reactive electrophiles introduced from

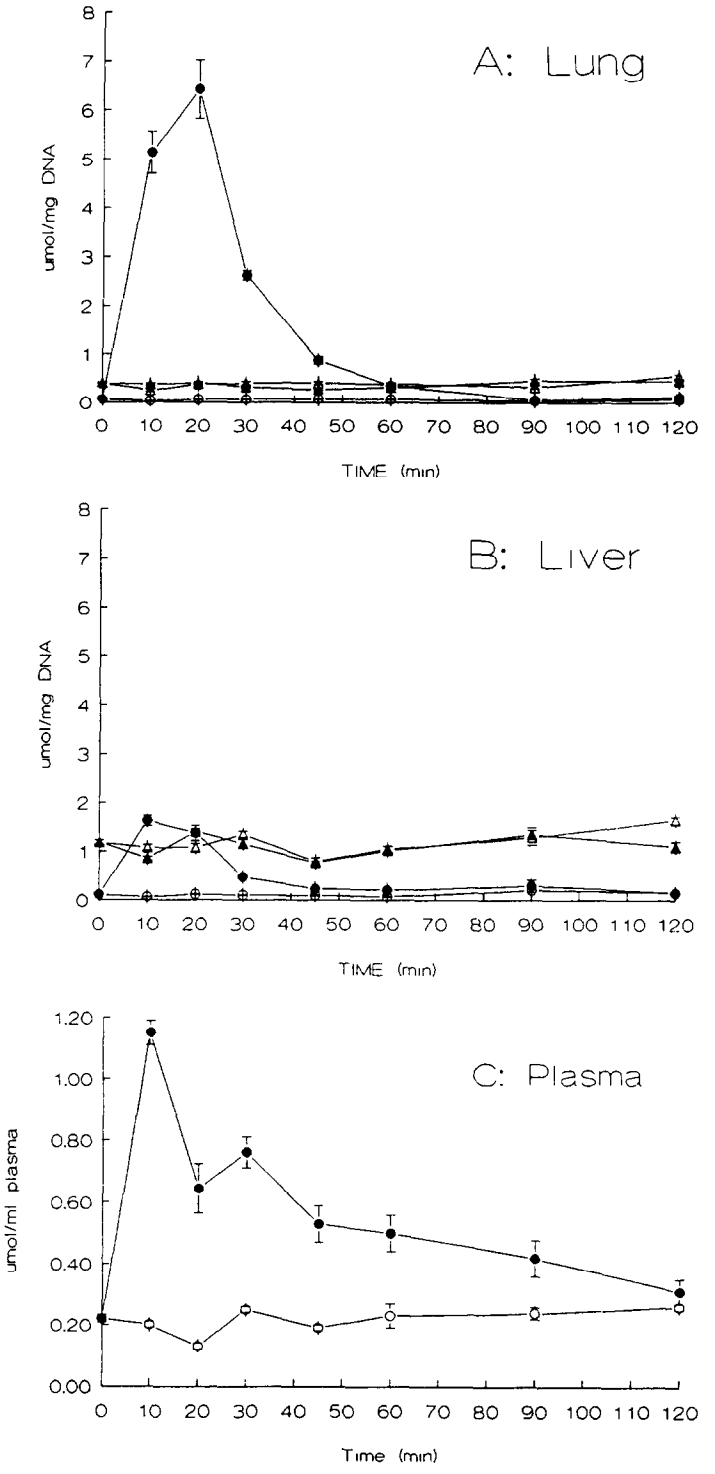

Fig. 4. Mean levels of CySH and GSH in the lungs (A) or livers (B) of four rats injected i.p. with 3 mmol/kg of CIPE or physiological saline. Saline controls: (○) CySH; (△) GSH. CIPE: (●) CySH; (▲) GSH. (C) Levels of NPSH in the plasma of the four rats used in (B). (○) Saline controls; (●) CIPE. Results in all cases are the means (± SE) of determinations in each of four rats killed at each time point.

the environment interact with these systems and in these reactions GSH may serve as either a nucleophile forming conjugates or as a reductant where it is oxidized to its disulphide. That depletion of cellular thiols by foreign chemicals may have serious consequences is shown by the hepatotoxicity induced by paracetamol [18] and the pulmonary toxicity of 4-ipomeanol [19] and butadiene [20]. Initial studies showed that the amount of both NPSH and GSH in lung was reduced by over one third in animals exposed to PFIB. As different cell types within the lung are known to have different levels of, and differing capacities to synthesize, GSH [21], it is possible that not all cells need to be depleted of thiol for a toxic event to occur.

To raise pulmonary thiol levels, esters of CySH were selected for investigation as the studies of Benesch and Benesch [22] had shown that at physiological pH the thiol group of the esters of CySH ionized to a markedly greater extent than those of either CySH or GSH (16, 6 and 1%, respectively) so that on a molar basis a greater proportion of the nucleophile was available for reaction with a toxic electrophile. In addition, as shown by Servin *et al.* [23], lipid soluble esters might be more readily distributed to the lungs. All selected esters, except CTBE, were effective protectants with no apparent side effects at the doses used when given 30 min before exposure to PFIB. When given after exposure to PFIB all the esters were ineffective. NAc and CySH also protected against the lethal effects of PFIB but the animals were in poorer condition.

Reduction of the levels of thiols in rats by pretreatment with BSO, an inhibitor of γ-glutamyl cysteine synthetase, causes maximal reduction of intracellular GSH in liver within 2 hr of its administration [24, 25]. In the lung, however, this does not occur for at least 10 hr [26, 27]; a measure of the much slower overall turnover of GSH in this organ. When rats pretreated with BSO were exposed to PFIB 16 hr after pretreatment they were found to be more susceptible to the effects of PFIB with earlier signs of respiratory distress and greater lethality. Administration of NAc, CySH and the cysteine ester to rats pretreated with BSO reduces the toxic effects of PFIB and demonstrates that cells depleted of GSH may be protected if sufficient amounts of other nucleophiles, such as CySH, can be generated within target cells.

Measurement of the changes in plasma and pulmonary thiols confirmed that after CME and CDME administration the ester increased plasma levels of NPSH up to 600%, and maximum pulmonary levels by several thousand per cent though little of the parent ester was detected. In contrast, although CySH and NAc protected against PFIB they only raised pulmonary thiol levels by 100–160%. More detailed investigation using CIPE showed that the levels of CySH in the lung greatly exceeded those of GSH, which remained unchanged, whereas the hepatic levels of CySH increased only to those of GSH. It is apparent that whilst both CySH and, in particular, NAc raise plasma levels of NPSH only the esters markedly increase tissue levels of CySH presumably as a result of their increased lipophilicity, and ready penetration of cell membranes.

The mechanism by which these esters preferentially raise tissue CySH but not GSH levels is at present unresolved. Servin *et al.* [23] showed that [^{35}S]CEE was distributed rapidly throughout the body; therefore, following absorption the esters are transported via the blood to the tissue and on entry into cells are hydrolysed by endogenous carboxyesterases. The efficacy of individual esters may reflect the amount and substrate specificity of tissue carboxyesterases; for example, CTBE may not be hydrolysed and, therefore, no protection is achieved. Alternatively, the rate of metabolism of CySH in each tissue may differ such that levels are sustained longer in the lung than in the liver. Preliminary experiments in this laboratory support these hypotheses in that CIPE is hydrolysed and CySH is metabolized more slowly in rat lung homogenates than in liver.

The lack of change in the GSH levels may reflect the transience of the elevated CySH levels or that the normal feedback inhibition of γ-glutamyl synthetase prevents the exploitation of the elevated levels of CySH by the cell.

These esters and their derivatives have an important role to play in the study of mechanisms of protection against inhaled toxicants, particularly those which elicit their major effect within the lung itself. Selective increase in the levels of CySH, may increase the level of protection by "titrating out" the incoming electrophile before damage to other cell constituents ensues. The possibility exists that a broad spectrum protective pretreatment might be developed which may protect simultaneously against the toxic effects of other gaseous electrophiles and toxicants such as phosgene. Increased levels of CySH and GSH in the epithelial lining fluid of airways may also be an important part of the protective mechanism as levels have been shown to be modified by inhaled toxicants, and increased by NAc administration [28].

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