Carcinogenesis vol.11 no.5 pp 755–760, 1990

Analysis of styrene oxide–globin adducts based upon reaction with Raney nickel

David Ting, Martyn T.Smith, Penelope Doane-Setzer and S.M.Rappaport[1]

Department of Biomedical and Environmental Health Sciences, School of Public Health, University of California, Berkeley, CA 94720, USA

[1]To whom correspondence should be addressed

A new method has been developed for determination of styrene oxide–globin adducts. The technique takes advantage of the reaction between alkylated globin and Raney nickel, which cleaves the carbon–sulfur bond in the styrene oxide–cysteine adduct to form 1-phenylethanol (1-PE) and 2-phenylethanol (2-PE). These alcohols are then reacted with pentafluorobenzoyl chloride and analyzed by GC–ECD. The method appears useful for biological monitoring of individuals exposed to styrene and, potentially, to other chemicals or their electrophilic metabolites which can react with cysteine residues in available proteins. The detection limit of the method, which is 0.04 nmol adducts/sample, indicates that it should be possible to detect adducts in the blood of people who are occupationally exposed to at least 18 mg/m^3 of styrene. Analysis of globin from human whole blood which had been modified with [^{14}C]styrene oxide indicated that 6% of the total globin adducts were detected. The method was applied to human and rat blood which had been treated with styrene oxide *in vitro* and to blood from rats given a single i.p. dose of styrene *in vivo*. Results from these experiments indicate that 77 times more adducts were detected at a given dose from rat globin than from human globin and that only 0.015% of the styrene dose was bioavailable as styrene oxide in the blood of rats. The reaction with Raney nickel is conducted at 5°C to minimize unfavorable side reactions, such as degradation of 1- and 2-PE and conversion of styrene glycol to 1- and 2-PE. The optimal amount of Raney nickel was found to be 5–6 g/g globin. Since the recovery of 1-PE was not reproducible, only 2-PE is used for quantitation.

Introduction

Alkylating agents, either direct-acting or generated *in vivo*, form an important class of environmental mutagens and carcinogens. These agents are characterized by their ability to bind covalently to nucleophilic sites in proteins and DNA. Ehrenberg, Osterman-Golkar and co-workers (1–3) first proposed the use of hemoglobin as a surrogate for DNA in the estimation of *in vivo* alkylation. Reactions between alkylating agents and various amino acid residues in the globin (Gb*) chain of the hemoglobin molecule form adducts which can be isolated and measured. The choice of hemoglobin has the following advantages: blood samples are easily acquired from humans; red blood cells (RBC) contain large amounts of Gb (~0.14 g/ml); and there is no known repair system for Gb adducts. Since RBC remain in the circulation for ~120 days in humans, Gb adducts can be used for the determination of cumulative doses over several months. In addition, animal studies hhave indicated that, for a number of chemicals, levels of Gb adducts are proportional to DNA adducts (4,5).

A number of instrumental methods have been reported for measuring Gb adducts in human populations. The first of these employed acid hydrolysis of Gb, followed by multiple chromatographic steps to isolate the alkylation products and finally the use of GC–MS to detect the adducts of histidine (2) or cysteine (6). Such techniques were extremely time consuming, however, and gave way to more streamlined methods which were specific for particular types of adducts. The most widely used technique employs base-catalyzed hydrolysis to regenerate 4-aminobiphenyl and other aromatic amines from cysteine adducts of Gb (4,7). Unfortunately, this procedure can only be applied to substances which produce arylnitroso-metabolites. Tornqvist and co-workers (8,9) modified the Edman degradation procedure to determine adducts of the N-terminal residue, valine, and applied the method to detect ethoxylated valine in humans exposed to ethylene oxide.

Here, we report an alternative method for the determination of Gb adducts that is sensitive, specific and relatively simple to apply. It is based on the well known desulfurization of sulfur-containing compounds by Raney nickel (Ra-Ni) (10,11) in which the carbon–sulfur bond is cleaved between the alkylating agent and cysteine and at least one new carbon–hydrogen bond is formed. Although this paper deals exclusively with application of the Ra-Ni technique to styrene oxide–Gb adducts, the method can, in theory, be used to cleave virtually any cysteine adduct from either Gb or other proteins.

While applications of the Ra-Ni method to the analysis of adducts from Gb or other blood proteins have apparently not been reported, Pachecka *et al.* (12) demonstrated that when styrene oxide–glutathione adducts were refluxed with Ra-Ni in ethanol, 1-phenylethanol (1-PE) and 2-phenylethanol (2-PE) were produced. This reaction between cysteine-bound styrene oxide and Ra-Ni is shown in Figure 1.

Materials and methods

Modification of human and rat blood in vitro

A solution of styrene oxide or labeled styrene oxide (from Aldrich or [^{14}C]styrene oxide from Amersham, diluted to a sp. act. of 1.5 mCi/mmol with unlabeled styrene oxide) in acetone was mixed with at least 10 volumes of physiological saline. Fresh whole blood was then added and the solution was mixed and incubated at 37°C for 2 h. Human blood was obtained from a single donor. Rat blood was obtained by cardiac puncture (4–5 ml/animal); since this blood was not pooled, each measurement represented a single rat.

Modification of rat blood following administration of styrene in vivo

Male Sprague–Dawley rats (three per group) weighing 300 g were given a single i.p. injection of styrene in corn oil. Doses were as follows: 0, 0.5, 1, 2 and 3 mmol/kg. Blood was obtained by cardiac puncture 20 h after dosing. Significant clotting occurred in the blood obtained from one animal in each of the first three dose groups. It was not possible to analyze the blood from these animals.

Isolation of Gb from blood

After the removal of plasma, the red blood cells (RBC) were washed three times with several volumes of physiological saline. The RBC were lysed by the addition

*Abbreviations: Gb, globin, Ra-Ni, Raney nickel; 1-PE, 1-phenylethanol; 2-PE, 2-phenylethanol; RBC, red blood cells; PFB, pentafluorobenzoyl, CI, chemical ionization; EI, electron-impact ionization.

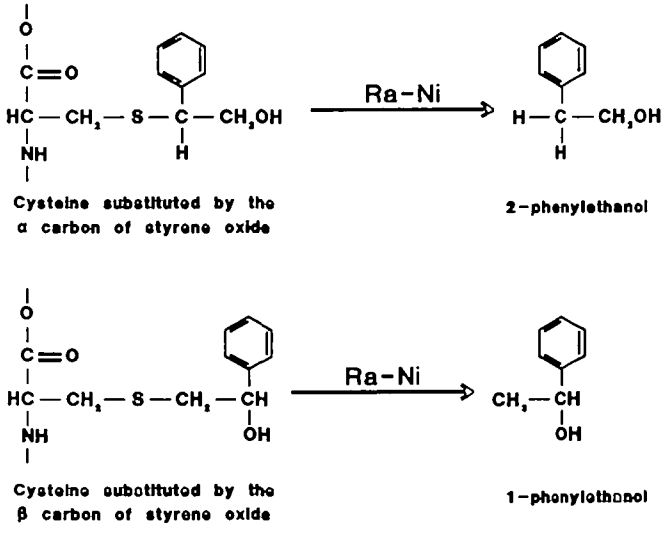

Fig. 1. Cleavage of styrene oxide–cysteine by Ra-Ni

of 2 vol deionized water and the hemolysate was centrifuged at 21 000 *g* at 5°C for 20 min to precipitate the cell membranes. The hemoglobin solution was added dropwise to 10 vol of cold 1% HCl in acetone to precipitate the Gb (13). The Gb was filtered, washed with several ml of acetone, dissolved in 10 ml of 0.1 M NaOH and dialyzed exhaustively at 5°C against distilled deionized water or 0.02 M Tris–HCl buffer in 0.6% NaCl, pH 7.2. The Gb was then precipitated by the method above and dried in a vacuum dessicator to constant weight.

Scintillation counting

The Gb sample was dissolved in 5–10 ml of 0.5 M NaOH. 0.1 ml of this solution was mixed with 5 ml scintillation fluid (Ecolite from WestChem) and counted for 10 min with a Beckman LS 3801 scintillation counter

Cleavage of styrene oxide–Gb adducts by Ra-Ni

The Gb sample was dissolved in 5–10 ml of 0.5 M NaOH and the pH adjusted to 7.8 with dilute HCl. The solution was stirred and heated to 80°C for 25 min to denature the protein. After cooling to room temperature, Protease XXV (from Sigma, ~7% W/W of the Gb) was added to the sample. The sample was digested at 37°C for 6 h. Then 3–4 ml of 1 M NaOH and 2 ml of acetone were added to the solution so that the final pH was >13. The sample was then spiked with 100 ng of 3-phenyl-1-propanol, which served as an internal standard After cooling the sample solution to 5°C, cold Ra-Ni (50% slurry, from Aldrich, 5–6 g per g of Gb) was added. This suspension was shaken at 5°C for 40 min Upon completion of the reaction, Ra-Ni was allowed to settle (~10 min). The supernatant was transferred to a glass tube and extracted twice with 6 ml of ethyl ether. The ether layers were combined and reduced to ~1 ml under a stream of nitrogen. This solution was washed twice with 1 ml of 0.1 M HCl and then reduced almost to dryness under a stream of nitrogen.

Derivatization of 1-PE and 2-PE with pentafluorobenzoyl (PFB) chloride

The residue of the Ra-Ni reaction was dissolved in 0.5 ml of hexane. Then 3 μl of pyridine and 1.5 μl of PFB chloride (Aldrich) were added and the solution was warmed to 50°C for 20 min. Excess reagents were removed under nitrogen. The residue was dissolved in 0.5 ml of 85% methanol and extracted with 0 5 ml hexane (14).

Chromatographic analysis

Gas chromatography was performed with a Varian 3700 chromatograph equipped with an electron-capture detector. A 15 m × 0.32 mm i.d., fused-silica column was used (DB-5; 1.0 μm film thickness; from J. and W.Scientific). The carrier gas was helium with a linear velocity of 29 cm/s. The injection volume was 2 μl, with a split ratio of 20:1. (A splitter was used to minimize interference from late eluting peaks with retention times >40 min. In a few samples with large concentrations of 2-PE-PFB a split ratio of 50:1 was used.) The injector and detector temperatures were 210 and 280°C, respectively [Although a detector temperature of 280°C was employed for these experiments, subsequent work has shown that an ~2-fold increase in sensitivity is possible by reducing the temperature to between 210 and 240°C. The peak-height ratio of 2-PE-PFB to the internal standard was found to be constant at 1.14 ± 0.05 (mean ± SD) between 210 and 320°C] The analysis was performed isothermally at a column temperature of 180°C.

Gas chromatography–mass spectrometry analysis employed a Hewlett-Packard 5985B GC–MS system with a fused-silica column (DB-5; 0.25 μm film thickness; 0.25 mm i.d. × 30 m) with a sequential temperature program of 80°C for 3 min, followed by an increase of 10°C/min to 300°C. Helium was used as the carrier gas (30 cm/s). Both chemical ionization (CI) using methane (0 8 Torr) as the reagent gas and electron-impact ionization (EI) (70 eV) were used.

Determination of recovery

Quadruplicate aliquots of solutions of 2-PE containing 0.42, 0.84 and 1 30 nmol per sample were reacted with Ra-Ni at a pH >12 The samples were treated and analyzed as above and the peak-height ratios of the 2-PE-PFB and 3-phenyl-1-propanol-PFB were compared with those of analytical standards.

Conversion of styrene glycol to 2-PE by Raney nickel

Different amounts of styrene glycol (Aldrich) were added to a solution containing 10 ml of 0.1 M phosphate buffer at pH 7.4, 0.5 ml of 1 M NaOH, 2 ml of acetone and 10 μl (20 μg/ml) of 3-phenyl-1-propanol. After the addition of Ra-Ni (0.2–0 5 g), the mixture was shaken at 5, 25 or 55°C for 40 min. The 2-PE was extracted with ethyl ether and analyzed as described above

Table I. Conversion of styrene glycol to 2-phenylethanol by Raney nickel

Temperature (°C)	Styrene glycol (nmol/sample)	Conversion to 2-PE (% mol/mol)[a]
55	811	0.17 ± 0 08 (3)
25	811	0.11 ± 0 03 (3)
5	1622	0.05 ± 0.01 (3)

Results are estimated mean ± SD and number of replicates for each group.
[a]Results not corrected for recovery.

Results

Optimization of reaction

Recovery. The recoveries (mean ± SE for $n = 4$ per group) observed when 2-PE was reacted with Ra-Ni at 5°C for 40 min and carried through the above procedure were 86.0 ± 2.8% at 0.42 nmol/sample, 79.4 ± 1.2% at 0.84 nmol/sample, and 79.2 ± 1.6% at 1.3 nmol/sample. Since a one-way analysis of variance could detect no difference in the recoveries at a significance level of 0.05, the data were pooled to yield an overall recovery of 81.6 ± 1.2% ($n = 12$). The results indicate that 2-PE is relatively stable under the conditions which lead to the cleavage of the styrene oxide–cysteine adduct. Since the recovery of 1-PE-PFB was not found to be reproducible, only 2-PE-PFB was used for quantitation.

Temperature. Even after exhaustive dialysis it is possible that small amounts of styrene glycol are still absorbed onto the Gb. We found that the ability of Ra-Ni to cleave the styrene oxide–Gb adducts was not affected by temperature between 5 and 55°C (data not shown). However, reaction between Ra-Ni and styrene glycol was found to be less efficient at 5°C (Table I); thus, all experiments were conducted at 5°C.

Amount of Ra-Ni. Styrene oxide (1.6 μmol) was incubated with 15 ml of fresh human whole blood. Globin was isolated and divided into 0.1 g portions. After adding different amounts of Ra-Ni to each portion, the samples were reacted at 5°C for 40 min. The results, shown in Figure 2, indicate that the amount of 2-PE released from the reaction increased rapidly with the amount of Ra-Ni added up to ~5 g Ra-Ni/g Gb, after which significantly greater yields were not obtained. Thus, the amount of Ra-Ni used in subsequent experiments was 5–6 g/g Gb.

GC–MS analysis of 2-PE-PFB

The result of GC–MS analysis of a 2-PE-PFB standard is shown in Figure 3. Under the mass spectrometry conditions, 2-PE-PFB was very labile and no parent peak (316 *m/z*) was observed in either CI or EI modes. Only one major ion was observed in the CI spectrum at 105 *m/z*; this corresponds to the molecular ion

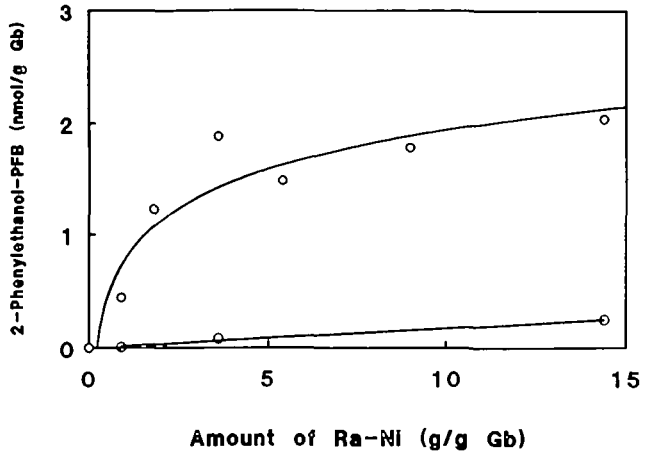

Fig. 2. Closed circles show the yield of 2-PE from styrene oxide-modified globin versus the amount of Ra-Ni added. Open circles represent controls

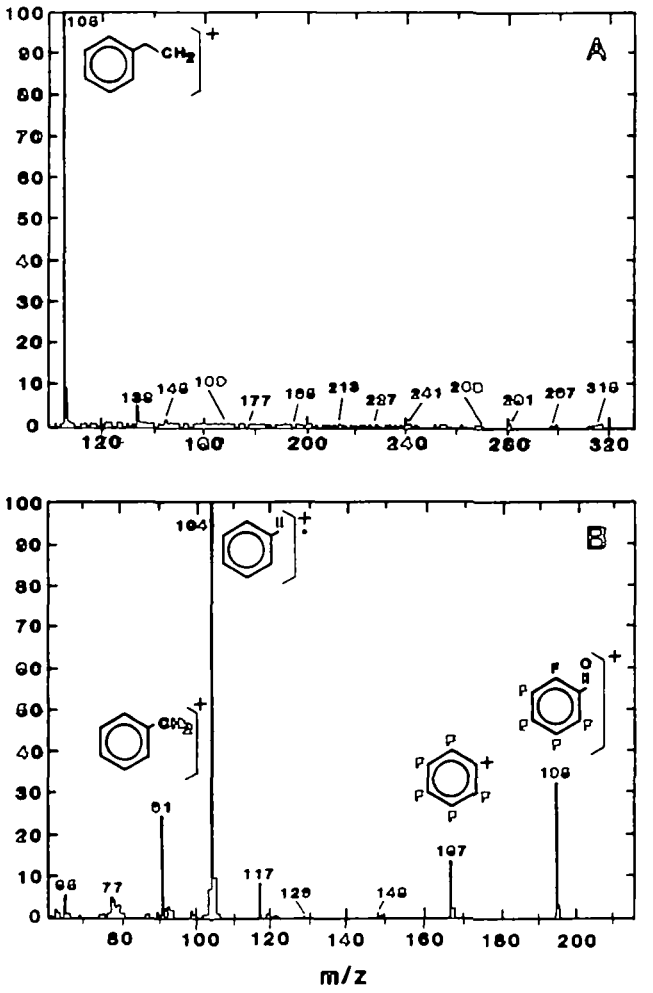

Fig. 3. Mass spectra obtained from GC–MS analysis of an analytical standard of 2-PE-PFB with identification of major fragment ions. (A) Spectrum from chemical ionization; (B) spectrum from electron-impact ionization

less a fragment of 211 *m/z*, corresponding to C_6F_5COO. This product would be expected from 2-PE-PFB due to the lability of the ester bond in that molecule. In the EI spectrum, expected fragments from 2-PE were detected at 91 (C_7H_7) and 104 (C_8H_8) *m/z* respectively. These spectra, while not providing unambiguous confirmation of the structure, are consistent with the assumption that 2-PE is released from globin adducts of styrene oxide by the Ra-Ni reaction. This is supported by the observation of similar CI and EI spectra (not shown), from GC–MS of 3-phenyl-1-propanol-PFB which were obtained from derivatization of the internal standard.

Table II. Proportion of styrene oxide–globin adducts detected by the Ra-Ni method[a]

Styrene oxide concentration (μM)	% of total adducts detected
33	5.8 ± 0.6 (2)
50	4.8 (1)
82	6 1 ± 0.9 (3)
Total	5.8 ± 0.8 (6)

Results are estimated mean ± SD and number of replicates for each group. [a]Results are corrected for 81 6% recovery and assuming 60% of styrene oxide–cysteine adducts are alpha-substituted (12). Total adducts were determined by counting Gb which had been reacted with ^{14}C-labeled styrene oxide.

Comparison between total binding and the 2-PE released by Ra-Ni

Portions (5 ml) of fresh human whole blood were reacted with different amounts of ^{14}C-labeled styrene oxide. A small amount of the isolated Gb was removed for scintillation counting and the remainder was subjected to the Ra-Ni reaction. The results, given in Table II, indicate that ~6% of the total styrene oxide–Gb adducts, determined by counting, were detected by the Ra-Ni method. This fraction of the total was independent of the styrene oxide concentration between 33 and 82 μM.

One additional portion of blood, which had been reacted with 33 μM styrene oxide, was counted (5.5 nmol total binding) and carried through the analytical procedure without addition of Ra-Ni. This sample is shown as chromatogram A in Figure 4. Since 2-PE was not detected, it can be concluded that Ra-Ni is needed to release the adducts. For comparison, a blood sample which had been incubated with 82 μM of styrene oxide and then reacted with Ra-Ni is shown as chromatogram B in Figure 4.

Analysis of blood reacted with styrene oxide in vitro

Portions (5 ml) of fresh whole blood were reacted *in vitro* with styrene oxide at concentrations ranging between 27 and 340 μM. Following isolation of the Gb and the Ra-Ni analysis, the dose–response relationships were investigated. After correction for recovery, the human data were found to fit both a linear model (r^2 = 0.946) and a multiplicative model (r^2 = 0.947) about equally well. As shown in Figure 5A, it is reasonable to conclude that the amount of 2-PE detected in human globin was directly proportional to the concentration of styrene oxide between 27 and 318 μM, with a slope of 9.83×10^{-3} nmol 2-PE/g Gb/μM styrene oxide.

Rat blood was found to produce much greater amounts of 2-PE than human blood at a given concentration of styrene oxide. As shown in Figure 5B, the dose–response relationship was decidedly nonlinear at styrene oxide concentrations above 80 μM. In the dose region between 0 and 80 μM styrene oxide a significant linear relationship was observed (r^2 = 0.697; P = 0.039; n = 6) with a slope of 0.76 nmol 2-PE/g Gb/μM styrene oxide.

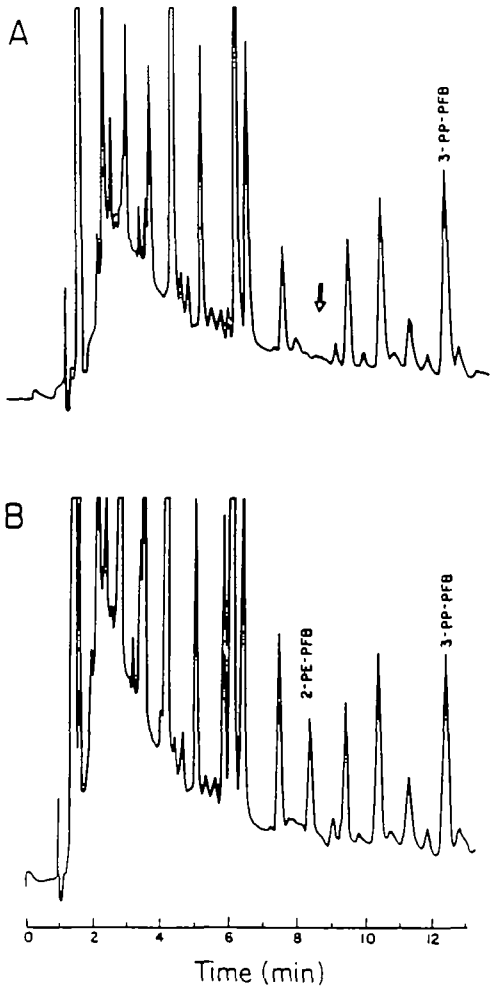

Fig. 4. Gas chromatograms of Gb samples from whole human blood modified with styrene oxide *in vitro*. (**A**) Sample containing 0.8 g Gb and incubated with 33 μM styrene oxide carried through the procedure without addition of Ra-Ni. Arrow indicates retention time for 2-PE-PFB. (**B**) Sample containing 0.9 g Gb incubated with 82 μM styrene oxide and carried through the procedure with addition of Ra-Ni.

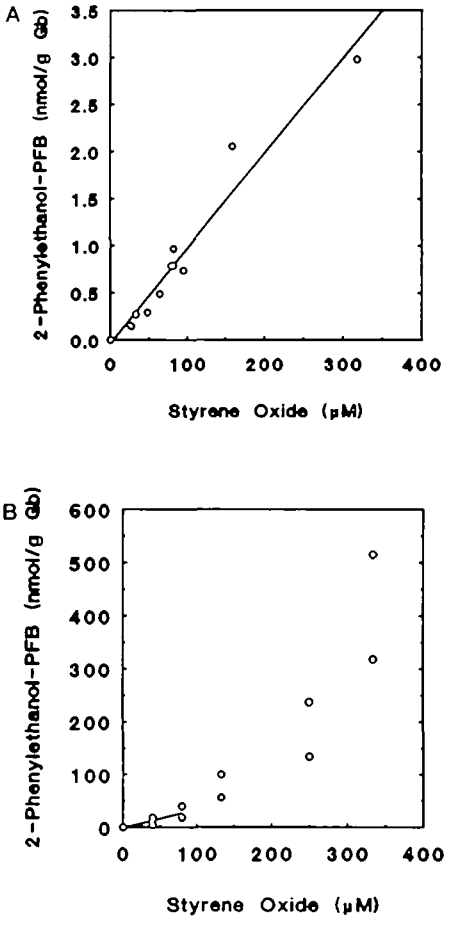

Fig. 5. Dose–response curves showing production of globin adducts in whole blood reacted with styrene oxide *in vitro* (Corrected for 81.6% recovery) (**A**) Human blood obtained from a single donor. (**B**) Rat blood, each point represents a single rat. The solid line indicates the linear region of the dose–response curve

Precision and detection limit

The precision of the method was estimated from the human *in vitro* data, which had been obtained from a single sample of blood, by partitioning the residual error from the regression analysis applied to the multiplicative model. In this case the mean square error in the dependent variable (amount of 2-PE) was 0.050 which corresponds to a geometric standard deviation of 1.25 and a coefficient of variation of 22.7%.

Since the multiplicative model can be applied to results obtained from this method, one can assume that the logarithms of measurements obtained from analysis at a particular level are normally distributed (15). Thus, the detection limit of 2-PE was estimated from seven independent blank determinations of human whole blood as $\exp[\bar{x}_L + 2s_L] = 0.04$ nmol 2-PE-PFB/sample (0.5–0.6 g Gb), where $\bar{x}_L$ and s_L are the mean and standard deviation, respectively, of the log-transformed blank concentrations. In other words, a measurement of 2-PE-PFB greater than 0.04 nmol/sample would be detected by chance in a blank sample with a probability of $<5\%$.

Analysis of blood from rats treated with styrene in vivo

Since styrene is metabolized to styrene oxide in the liver of many animal species including humans (16–19), we wished to demonstrate that this method can detect styrene oxide formed *in vivo*. Male Sprague–Dawley rats were treated with between 0.5 and 3 mmol styrene/kg body wt by i.p. injection. Upon analysis of the Gb isolated from these animals, a linear dose–response curve was observed (Figure 6), with a slope of 2.30 nmol 2-PE/g Gb/mmol styrene/kg body wt ($r^2 = 0.811$).

Discussion

The use of adducts of Gb, resulting from the reaction between nucleophilic amino acids and genotoxic species, represents an important tool for human dosimetry (1–9). However, only a technique which is sufficiently straightforward to allow processing of large numbers of samples will be useful as a tool for monitoring human populations exposed to genotoxicants. Our interest in monitoring the bioavailable dose of styrene oxide received by humans exposed to styrene led us to seek a simple alternative for monitoring styrene oxide–Gb adducts.

Hemminki (20) reported that styrene oxide, when reacted with polyamino acids *in vitro*, was ~40 times more reactive towards polycysteine than polyhistidine, polyserine or polylysine. He also showed that styrene oxide–cysteine was the predominant product of reaction between styrene oxide and human whole blood *in vitro* (21). We therefore undertook to develop a procedure which would allow us to selectively measure styrene oxide–cysteine adducts in blood proteins.

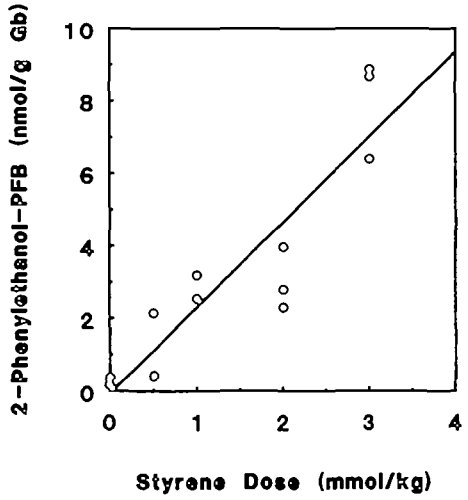

Fig. 6. Dose–response curve showing globin adducts in rats following a single i.p. dose of styrene. (Corrected for 81.6% recovery).

In developing such a method we were intrigued by the use of Ra-Ni to cleave styrene oxide–cysteine adducts from glutathione conjugates (12). We applied a modification of that procedure to release adducts from Gb which had been derived from human and rat blood, modified with styrene oxide *in vitro*, or from the blood from rats to which styrene had been administered *in vivo*. We observed in both cases that reaction of Gb with Ra-Ni yielded 2-PE, one product which would be expected to result from cleavage of styrene oxide–cysteine.

We demonstrated that by running the Ra-Ni reaction at 5°C, the interference can be minimized from residual styrene glycol which would be expected to be present in blood samples as the result of hydrolysis of styrene oxide. It is also known that in refluxing ethanol, Ra-Ni can convert 1-PE and 2-PE to toluene and ethylbenzene, respectively (22). By using a low reaction temperature and a short reaction time we tried to minimize this loss.

Although we showed that Ra-Ni could be successfully applied to cleave styrene oxide adducts from Gb our results indicate that only ~6% of the total binding to human globin was actually recovered as isomers of phenylethanol. This is in contrast to the results of Hemminki (21), who suggested that cysteine adducts of Gb constituted the primary products of reaction *in vitro* between styrene oxide and Gb in human whole blood. Our experiments indicate either that the Ra-Ni is rather inefficient at cleaving cysteine adducts from human Gb or that other nucleophilic sites in human Gb account for the bulk of reaction with styrene oxide. Further experimentation is required to resolve this question.

When the Ra-Ni procedure was applied to Gb from blood which had been reacted with styrene oxide *in vitro* it was observed that rat Gb released much more 2-PE than human Gb at a given concentration of styrene oxide. When the slopes of the linear portions of the two dose–response curves were compared, [(0.76 nmol 2-PE/g Gb/μmol styrene oxide/l rat blood)/(9.83 × 10^{-3} nmol 2-PE/g Gb/μmol styrene oxide/l human blood)] = 77 times more adduct was detected from the rat globin at a given dose of styrene oxide. This suggests either that the cysteine residues of rat globin are more accessible than those of human globin for reaction with styrene oxide or that Ra-Ni cleaves adducts more readily from rat globin than from human globin or both.

When styrene oxide was reacted with rat blood *in vitro* the slope of the dose–response curve was 0.76 nmol 2-PE/g Gb/μmol styrene oxide/l blood in the linear range of 0–80 μM of styrene oxide. Since the concentration of Gb in rat blood is ~0.14 g/ml, this slope corresponds to 110 nmol 2-PE/μmol of styrene oxide administered. The slope of the dose–response curve observed from styrene-treated rats was 2.28 nmol 2-PE/g Gb/mmol styrene/kg body wt. Since rat blood contains ~7 g Gb/kg body wt, this slope corresponds to 16.0 nmol 2-PE/mmol of styrene administered. Assuming complete metabolism of styrene to styrene oxide in the rat [only ~4% of the styrene dose is eliminated unchanged in the breath (23)], then the ratio of the slopes from these two experiments represents the bioavailable dose of styrene oxide in the systemic circulation of the rat following metabolism of styrene in tissues other than the blood. That is, (16.0 nmol 2-PE/mmol styrene)/(110 nmol 2-PE/μmol styrene oxide) = 0.15 μmol styrene oxide/mmol styrene. This indicates that ~1.5 × 10^{-4} mmol of styrene oxide was released to the blood per mmol of styrene administered. In other words, 0.015% of the administered dose of styrene constituted the bioavailable dose of styrene oxide in the red blood cells of rats.

If the metabolism of styrene oxide is similar in rats and humans, this result has important health implications since it suggests that only a small fraction of the inhaled dose would be available as styrene oxide in tissues other than the liver. Investigation of globin samples obtained from individuals whose styrene exposure is known can resolve this question.

The only other *in vivo* data concerning the bioavailability of styrene oxide were reported by Byfalt-Nordqvist *et al.* (19). They indicated, on the basis of i.p. administration of both styrene and styrene oxide in male mice, that the total production of adducts to hemoglobin/mmol of agent administered was about the same for both compounds. Our results, which indicate that only ~0.015% of the styrene dose actually forms adducts with Gb, are at odds with those of Byfalt-Nordqvist *et al.*. It is possible that metabolism of styrene oxide is much different in the liver of the mouse and the rat. Another potential explanation concerns the method of administration of styrene oxide in the mouse experiments. If significant reaction of styrene oxide took place in the peritoneal cavities of the animals, then only a small fraction of the administered dose might actually have reached the blood.

The working range of this method for monitoring styrene oxide–Gb adducts can also be considered in relation to the current exposure limit of 213 mg/m^3 enforced by the Occupational Safety and Health Administration (OSHA) (24). Assuming a 5 ml blood sample, the detection limit of 0.04 nmol 2-PE/person corresponds to a level of hemoglobin–cysteine adducts of 41.6 nmol/person in a standard man with 5.2 l of blood. Since Table II indicates that only 5.8% of the total hemoglobin adducts are released by the Ra-Ni procedure, at least 717 nmol/person of total hemoglobin adducts are required. We also found, from incubation of human blood with 14 nmol of [^{14}C]styrene oxide *in vitro*, that 4.0% of the binding occurred in the hemoglobin (unpublished observation). Thus, the detection limit corresponds to a level of total blood protein adducts of 17.9 μmol/person. Assuming that, as for rats, 0.015% of the styrene dose is available as styrene oxide in the blood of humans exposed to styrene, a dose of 119 mmol of styrene is required per sample. Finally, because individuals are chronically exposed to styrene, hemoglobin adducts accumulate over several months. Since the lifetime of red blood cells is 120 days in humans the corresponding time constant would be 60 days or 8.57 weeks. Assuming exposure for 8 h/day and 5 days/week, that 70% of

inhaled styrene is absorbed in the blood (23) and that the respiratory ventilation rate is 1 m^3/h, this method should detect Gb adducts in the blood of those exposed to at least 51.6 mg/m^3 of styrene in the air. Since this concentration is less than one-fourth of the current OSHA limit, the method should be immediately useful for biological monitoring. Future improvements in the technique should result in substantial increases in sensitivity which will allow even lower doses to be detected.

The Ra-Ni method for monitoring cysteine adducts has advantages over most existing techniques as a tool for monitoring humans exposed to styrene and many other genotoxic agents. We are currently determining whether the method can be adapted for measuring styrene oxide–cysteine adducts in albumin as well. Eventually we plan to use the Ra-Ni methods to measure protein adducts in the blood of humans exposed to styrene and other genotoxic agents.

Acknowledgements

The authors wish to thank J.Woodlee for his assistance in developing the chromatographic method and Dr Robert Toia for performing the GC–MS measurements. This work was supported by grant RO1OH02221 from the National Institute for Occupational Safety and Health of the Centers of Disease Control, by grant P42ES04705 of the National Institute for Environmental Health Sciences and by the Health Effects Component of the University of California Toxic Substances Research and Teaching Program.

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Received on August 8, 1989; revised on January 12, 1990; accepted on February 9, 1990