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Biochemical Pharmacology 61 (2001) 493–501

Biochemical Pharmacology

Cell-specific loss of cytochrome P450 2B1 in rat lung following treatment with pneumotoxic and non-pneumotoxic trialkylphosphorothioates

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Received 25 January 2000; accepted 18 July 2000

Abstract

This study was designed to test the hypothesis that the reduction in cytochrome P450 (CYP) 2B1 content and activity of rat lung microsomes, following dosing with pneumotoxic trimethylphosphorothioates, results from damage to specific cell types. Of the lung cells exhibiting immunolabelling for CYP2B1, only type I cells showed signs of susceptibility to the pneumotoxins *O*,*O*.*S*-trimethylphosphorothioate and *O*,*S*,*S*-trimethylphosphorodithioate. While most type I cells became necrotic, type II and Clara cells showed no signs of injury, despite their gradual loss of CYP2B1, as detected by immunogold labelling. This loss of labelling was accompanied by a 75% reduction in the immunoreactive CYP2B1 content and an 85% reduction in pentoxyresorufin *O*-dealkylase activity in lung microsomes. In contrast, the non-pneumotoxic analogue *O*,*O*,*S*-trimethylphosphorodithioate, differing from *O*,*O*,*S*-trimethylphosphorothioate by only the presence of a P = *S* rather than a P = *O* moiety, caused an even more rapid fall in pulmonary pentoxyresorufin *O*-dealkylase activity, but only a slight reduction in the microsomal content of CYP2B1. The recovery of this activity began within 12 hr of dosing. *O*,*O*,*S*-Trimethylphosphorodithioate, which acts as a suicidal inhibitor of pulmonary CYP2B1, did not cause any detectable lung injury or increase in cell division. These results are consistent with the initial reduction in both enzyme content and activity caused by the P = *O* — containing pneumotoxins resulting, almost entirely, from death of type I cells. Subsequent reductions that occur long after clearance of the toxin may be exacerbated by the onset of mitosis in Clara and type II cells.

Keywords: Immunocytochemistry; Ultrastructure; Pulmonary; Bioactivation

1. Introduction

This study, to test the hypothesis that injury to a particular cell type is responsible for a dramatic pneumotoxin-induced fall in pulmonary CYP2B1 activity, compared the effects of three chemically closely related trimethylphosphorothioates (Fig. 1). The pneumotoxin TMPT1 has been identified as an impurity in malathion and phenthoate [1] and as a metabolite of dimethoate [2], one of the most important organophosphorus insecticides. It is activated by pulmonary CYP2B1 [3] and, like its homologue TMPT2, causes injury to type I pneumocytes [4]. Type I cells have been shown to express the activating enzyme [5,6] but equal, or greater, concentrations are present in Clara and type II cells [6]. Lung injury caused by chemicals that require metabolic activation by CYP enzymes usually involves damage to the non-ciliated (Clara) cells [7], resulting in a reduction in both the level and activity of pulmonary CYP, predominantly within the target cell population [8,9]. The lack of susceptibility of Clara and type II cells to injury by TMPT1 has been attributed to their ability to replenish toxin-depleted intracellular levels of glutathione by utilising extracellular reserves in the epithelial lining fluid [10]. This protective mechanism is not available to type I pneumocytes [11].

In contrast to the pneumotoxins described above, analogues, like TMPT3, which contain the P = *S* moiety have been shown to protect type I cells against pneumotoxicity [3]. This protection has been correlated with their particularly effective inhibition of pulmonary PROD, an indicator

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Abbreviations: CYP, cytochrome P450; TMPT1, *O*,*O*,*S*-trimethylphosphorothioate; TMPT2, *O*,*S*,*S*-trimethylphosphorodithioate; TMPT3, *O*,*O*,*S*-trimethylphosphorodithioate; and PROD, pentoxyresorufin *O*-dealkylase.

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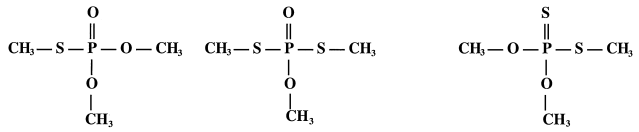
PII: S0006-2952(00)00572-4



TMPT1 TMPT2 TMPT3

Fig. 1. The molecular structure of TMPT1 (*O*,*O*,*S*-trimethylphosphorothioate) and TMPT2 (*O*,*S*,*S*-trimethylphosphorodithioate) compared with that of the non-pneumotoxic analogue TMPT3 (*O*,*O*,*S*-trimethylphosphorodithioate).

of CYP2B activity, despite their negligible effect on hepatic PROD activity [12]. Compounds such as TMPT3 probably act as suicide inhibitors of the activating enzyme [12,13]. The present study was designed to define the cellular localisation of CYP2B1 in the rat lung and evaluate the effects of its inhibition as opposed to cell injury. The heterogeneous nature of pulmonary tissue has necessitated the correlation of biochemical data, from pulmonary microsomes, with quantitative immunohistochemical studies of intact lung tissue.

2. Materials and methods

2.1. Chemicals

Pyrazole, pentoxyresorufin, Tween 20, and BSA were supplied by Sigma Chemical Co. Ltd. TMPT3 (99%) and TMPT1 (98%) were donated by Dr. J. Miles (Centers for Disease Control). TMPT2 (99%) was synthesised by Dr. P. Farmer (MRC Toxicology Unit). Purified CYP2B1/2 protein, together with a rabbit-derived polyclonal antibody to rat CYP2B1/2, was a gift from Dr. C.R. Wolf (ICRP Molecular Pharmacology Unit). A goat-derived polyclonal antibody to rat CYP2B1/2, manufactured by Daiichi Pure Chemical Co. Ltd., was purchased from Gentest. Fish gelatin and rabbit anti-goat immunoglobulin G, conjugated to colloidal gold, was purchased from British Biocell International.

2.2. Animals

Female outbred rats, LAC:P Wistar (160–200 g), supplied by the MRC Toxicology Unit, were given standard laboratory animal diet and water, *ad lib*., and maintained at 22 ± 2°, relative humidity 50–70%, and with a 12-hr light/dark cycle. Experiment 1: Rats (N = 32) were dosed by gavage with TMPT2 (25 mg/kg), in arachis oil, and killed at 2, 11, 24 and 72 hr after dosing. Control animals (N = 14) received an identical volume of solvent, by the same route, and were killed 24 hr after dosing. All were used for the estimation of PROD activity in lung microsomes. Experiment 2: Rats (N = 18) were dosed by gavage with TMPT1 (50 mg/kg), in arachis oil, and killed at 24 hr, 72 hr, and 8 days. Control animals (N = 6) received an identical volume of solvent, by the same route, and were killed 24 hr after dosing. All were used for immunoblotting and immunohistochemistry. Experiment 3: Rats (N = 58) were dosed by gavage with TMPT3 (14 mg/kg), in arachis oil, and killed at 2, 11, 24 and 72 hr and at 8 days after dosing. Control animals (N = 13) received an identical volume of solvent, by the same route, and were killed 24 hr after dosing. The lungs from 40 of these rats were used for immunoblotting and immunohistochemistry; the remainder were used for the estimation of PROD activity in lung microsomes.

2.3. Preparation of microsomal fractions

The entire lung (approximately 900–1000 mg) was homogenised in 4 volumes of ice-cold Tris/KCl buffer (0.05 M Tris, 0.154 M KCl adjusted to pH 7.6 with HCl) using an Ilado ×1020 homogeniser for 30 sec. Tissue homogenates were centrifuged for 15 min at 10,000 *g*, and the supernatant decanted and centrifuged for 60 min at 100,000 *g*. The final pellet was resuspended in Tris buffer and the protein content determined [14] using BSA as standard.

2.4. Immunoblotting

Microsomal proteins (22.5 μg) were separated by electrophoresis, from washed microsomal preparations, on a 9% (w/v) SDS polyacrylamide gel. They were blotted onto nitrocellulose membranes and incubated with the primary antibody diluted (1:500–1:3000) in Tris-buffered saline for 60 min at room temperature [12]. Bound antibodies were labelled with secondary antibodies conjugated to alkaline phosphatase. This activity was visualised using 5-bromo-4-chloro-3-indolylphosphate as substrate and nitroblue tetrazolium as the chromogen. The density of the immunoreactive bands was measured with an LKB 2202 Ultrascan laser densitometer.

2.5. CYP enzyme assays

Measurement of the dealkylation of pentoxyresorufin was carried out fluorimetrically on the day of microsomal preparation [15,16] using a substrate concentration of 10 μM.

2.6. Lung pathology

Three rats were taken, at random, from each of the following seven treatment groups: arachis oil (control), TMPT1 (24 and 72 hr), and TMPT3 (2, 11, 24, and 72 hr). Each rat was killed with CO_2. The left lung was removed and slices 500– to 600-μm thick were prepared using a McIlvain tissue chopper.

Slices for morphological examination were fixed overnight at 4° in 2% glutaraldehyde in buffer, 0.1 M with

respect to sodium cacodylate (final pH 7.3), and rinsed with the cacodylate buffer. They were subsequently post-fixed for 2 hr in 1% osmium tetroxide in buffer, 0.1 M with respect to sodium cacodylate and 0.04 M with respect to potassium ferrocyanide. All osmium-fixed samples were stained in 2% aqueous uranyl acetate for 18 hr at 60°, dehydrated through a series of ethanols, and embedded in Araldite. Semithin (2-μm) sections were stained with toluidine blue and examined to select areas of the proximal alveolar region [17] for ultramicrotomy. Ultrathin sections were stained with lead citrate and examined in a Zeiss 902A electron microscope. The absence of any detectable lung injury or infection was confirmed in control animals from each experimental group.

Slices for immunohistochemical examination were fixed for 30 min at room temperature in 4% formaldehyde, freshly made up from paraformaldehyde in Sorensen's phosphate buffer, final pH 7.3. Fixation was followed by three 30-min changes of Sorensen's phosphate buffer, pH 7.3, containing 3.5% sucrose (w/v), and overnight storage at 4° in the same buffer containing 3.5% sucrose (w/v) and 0.5 mM $CaCl_2$. The samples were dehydrated in 50% (v/v) ethanol on ice and transferred to LR gold resin (London Resin Company). Samples, in gelatin capsules, were polymerised using UV radiation (360 nm) for 24 hr at −25°. Semithin sections, stained with toluidine blue, were used to select areas of the proximal alveolar region for ultramicrotomy [17]. Ultrathin sections were cut from at least 2 blocks from each rat and collected on formvar-coated grids.

Sections were incubated with the primary antibody to rat CYP2B, diluted 1:100, for 16 hr at 4°. Control sections, using normal goat serum, were included in all incubations. All sections subsequently received three 120-min washes before incubation with 10 nm colloidal gold/rabbit anti-goat immunoglobulin G, diluted 1:50, for 16 hr at 4°. All incubations and washes were carried out in PBS containing 5% (w/v) BSA, 0.5% (v/v) fish gelatine, and 0.1% (v/v) Tween 20. All sections were stained with 4% (w/v) aqueous uranyl acetate for 30 min at room temperature before examination in a Jeol 100-CXII electron microscope.

Profiles of type II and Clara cells, which included the nucleus, were selected at random and recorded at an instrument magnification of ×10,000. At least 5 micrographs were recorded from each grid and printed to give a final magnification of ×30,000. The number of particles/μm^2 overlying the cytoplasm and nucleus, in each profile, was measured using a Summagraphics MM1103 digitising pad and "Vids V" software (Synoptics). The ratio of these counts was recorded for each profile.

2.7. Statistics

Biochemical results and preliminary immunohistochemical data are expressed as the mean ± SEM, except where specified otherwise. The preliminary immunohistochemical data were obtained from one grid from each of 3 animals in each treatment group; immunolabelling was determined over 5 type II and 5 Clara cells from each grid. More detailed immunohistochemical data were obtained using 2 further tissue blocks from each animal. One grid from each of these blocks was incubated with the anti-CYP2B antibody and another was incubated, in parallel, with non-immune serum. Five type II and 5 Clara cells were assessed for immunogold labelling. The data from the three main treatment groups were analysed as a "split-plot analysis of variance", with the whole plots being the individual rats and the split plots being the antibody incubations carried out on sections from each rat. Data from control and treated samples were also compared using Tukey's test. All analyses were conducted using "Minitab 10".

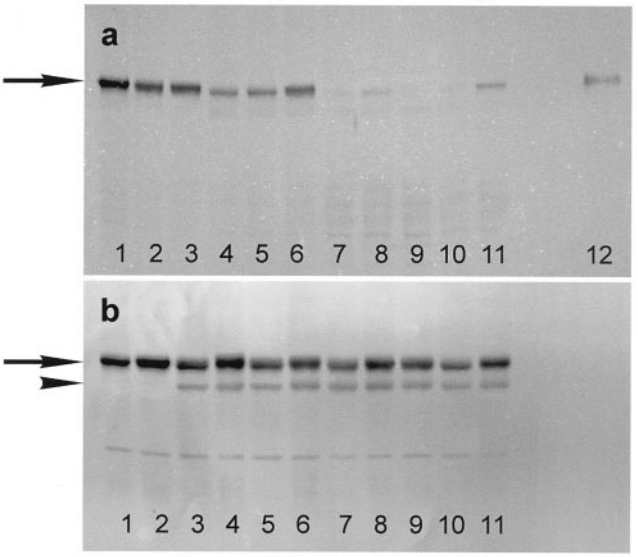


Fig. 2. **(a)** Effect of the pneumotoxin TMPT1 (50 mg/kg) on pulmonary CYP2B1 protein showing a labelled ~56 kDA band (arrows). Samples are from 1 day (lanes 4–6), 3 days (lanes 7–9), and 8 days (lanes 10 and 11) after treatment. Samples from untreated animals (lanes 1–3) and a CYP2B1 protein standard (lane 12) are included as controls, **(b)** Effect of the non-pneumotoxic analogue TMPT3 (14 mg/kg) showing the ~56 kDa band together with the development of a secondary band (arrowhead). Samples are from 2 hr (lanes 3–5), 11 hr (lanes 6–8), and 24 hr (lanes 9–11) after treatment. Samples from untreated animals (lanes 1 and 2) are included as controls.

3. Results

3.1. Effects of trimethylphosphorothioates on the pulmonary activity and concentration of CYP2B1

Western blots of pulmonary microsomes from control animals showed one band, which reacted with the primary antibody and co-migrated with the CYP2B1 standard, which has a molecular weight of 55.9 (Fig. 2a). Comparison with a dilution series of this standard indicated the presence of 23 ± 5 (N = 3) pmol protein in this band.

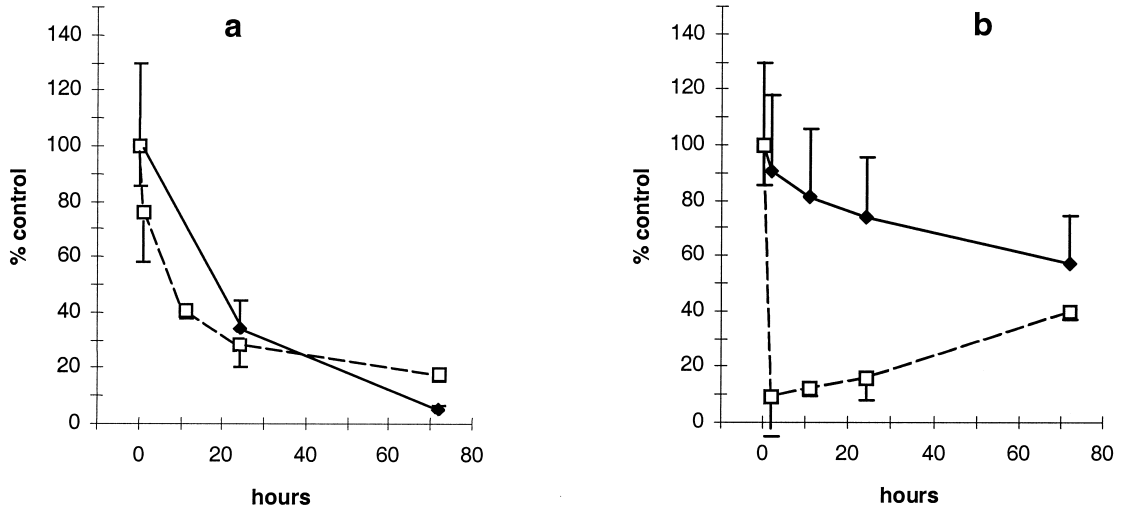


Fig. 3. Effect of (**a**) the pneumotoxin TMPT2 (25 mg/kg) and (**b**) its non-pneumotoxic analogue TMPT3 (14 mg/kg) on lung microsomal PROD activity (□) and protein levels (◆) of CYP2B1. All data are expressed as % control values ± SEM (N = 3 for protein samples and N = 5 for PROD samples, except at 11, 24, and 72 hrs when N = 4).

The PROD activity of control samples was found to be 21 ± 2 (N = 52) pmol/min/mg microsomal protein (results pooled with controls from parallel studies). Treatment with TMPT2 resulted in a fall in this PROD activity to 76% of control levels within 2 hr of dosing, and this decline continued until only 28% of the control level remained, at 24 hr (Fig. 3a). A further, but more gradual reduction in this activity continued until 72 hr after dosing (Fig. 3a). Immunoblots of microsomes from rats given an equitoxic dose of TMPT1 showed a parallel reduction in the CYP2B1 content which was clearly evident by visual inspection of the blots (Fig. 2a). Thus, both pneumotoxins produced a marked reduction in the level of CYP2B1 in pulmonary microsomes.

Treatment with TMPT3, the non-pneumotoxic analogue of TMPT1, resulted in a much less pronounced reduction in the immunolabelling of the CYP2B1 band (Fig. 3b). This decline, which continued until 72 hr after dosing, was invariably accompanied by the formation of a secondary immunoreactive band with a molecular weight approximately 5 kDa less than the constitutive protein (Fig. 2b). Despite the minimal effect of the non-pneumotoxic analogue on CYP2B1 content, the PROD activity of these microsomes fell rapidly, to 9% of control levels, within 2 hr of dosing, and subsequently began a slow recovery (Fig. 3b).

3.2. Effects of trimethylphosphorothioates on lung pathology

As shown in earlier studies [4,10], treatment with TMPT1 resulted in necrosis of most type I cells within 24 hr of dosing (data not shown). In the current study, only a few remnants of type I cells were evident 24 hr after treatment with TMPT1 or TMPT2, but other lung cells showed no signs of injury. All debris from damaged type I cells was cleared by 72 hr, but the numbers of intact type I cells remained low. Other lung cells were still devoid of any signs of injury at this time, but many type II cells showed signs of mitosis, as did several Clara cells, despite the absence of detectable injury to the bronchiolar epithelium. Very few of the characteristic electron-dense cytoplasmic granules were present in Clara cells 24 hr after dosing, but their numbers were normal at 72 hr.

In samples from animals treated with TMPT3, the non-pneumotoxic analogue, there was a slight increase in the number of electron-lucent granules in the cytoplasm of Clara cells, but no abnormalities or signs of increased mitotic division were detected.

3.3. Effects of trimethylphosphorothioates on the cellular distribution of CYP2B1

Immunogold labelling of lung tissue from untreated rats was largely restricted to the cytoplasm of type I and type II alveolar cells (Fig. 4) and also to the Clara cells of the bronchioles (Fig. 5). Few particles were present over the nucleoplasm of any type of cell. Preliminary studies demonstrated a consistent level of labelling over the cytoplasm of type II cells (4.1 ± 1.3 grains/μm^2, N = 15) and Clara cells (12.7 ± 2.4 grains/μm^2, N = 15), which was significantly higher than that observed after primary incubation with control serum (1.2 ± 0.5 grains/μm^2, N = 15). The labelling over type I cells was less consistent and often concentrated over small regions of the cytoplasm (Fig. 4c) and was usually found to be 40–80% of that over adjacent type II cells.

Immunohistochemistry of samples taken 72 hr after rats were dosed with TMPT3, when labelling of the ~56 kDa band was minimal (Fig. 2b), demonstrated an unexpectedly high density of gold particles over the cytoplasm of type II

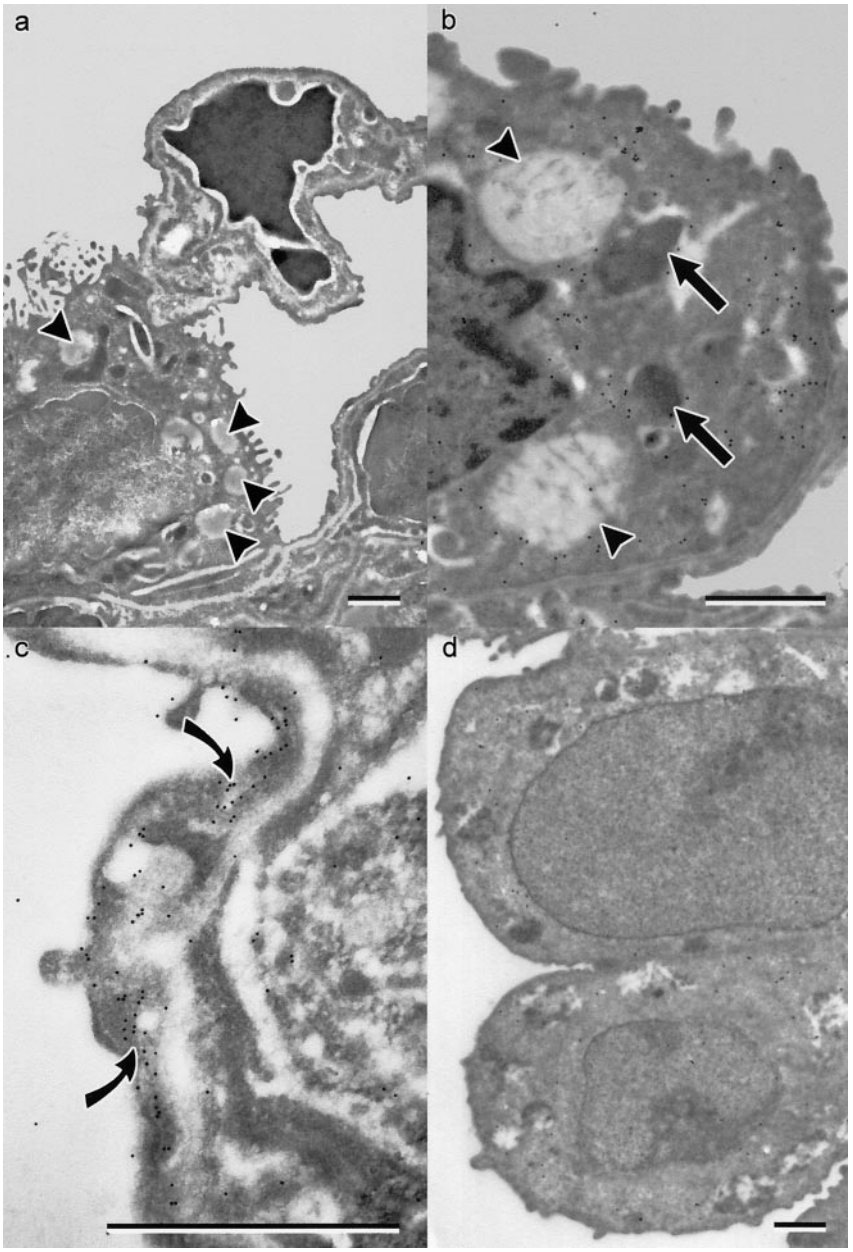


Fig. 4. Immunohistochemical localisation of CYP2B1 in alveolar tissue, (**a** and **b**) Type II cells from untreated rats show excellent preservation of ultrastructure except for the contents of lamellar bodies (black arrowheads). Immunogold labelling is largely cytoplasmic and absent from lamellar bodies (black arrowheads) or mitochondria (black arrows), (**c**) Type I cell from an untreated rat shows concentration of label over small regions of the cytoplasm (curved black arrows), (**d**) Two type II cells, 72 hr after treatment with TMPT1 (50 mg/kg), show signs of recent mitosis but little immunogold labelling, [all bars = 1 μm].

cells (4.4 $\pm$ 0.7 grains/μm^2, N = 15) and Clara cells (14.1 $\pm$ 3.3 grains/μm^2, N = 15). As these results were indistinguishable from controls, no further immunohistochemical studies were undertaken with any samples from TMPT3-treated rats. In contrast, lung sections from rats dosed with TMPT1 showed a dramatic reduction in immunolabelling. Few intact type I cells remained, even 24 hr after dosing, but the preservation of both type II and Clara cells was suitable for a detailed, quantitative investigation of the changes caused by this pneumotoxin.

Analysis of the preliminary data (not shown) from this larger study indicated the presence of slight inconsistencies between grids in the overall level of labelling. The intensity of labelling over the cytoplasm of type II and Clara cells was therefore normalised by reference to that over the nucleoplasm of the same cell. This technique was not adopted for type I pneumocytes as the nuclei of these cells were rarely present in the microscope field. The normalised data (Table 1) indicated a slight reduction in the immunodetectable content of CYP2B protein in the cytoplasm of type II cells within 24 hr of dosing with TMPT1, and a more pronounced reduction after 72 hr. Cells showing signs of

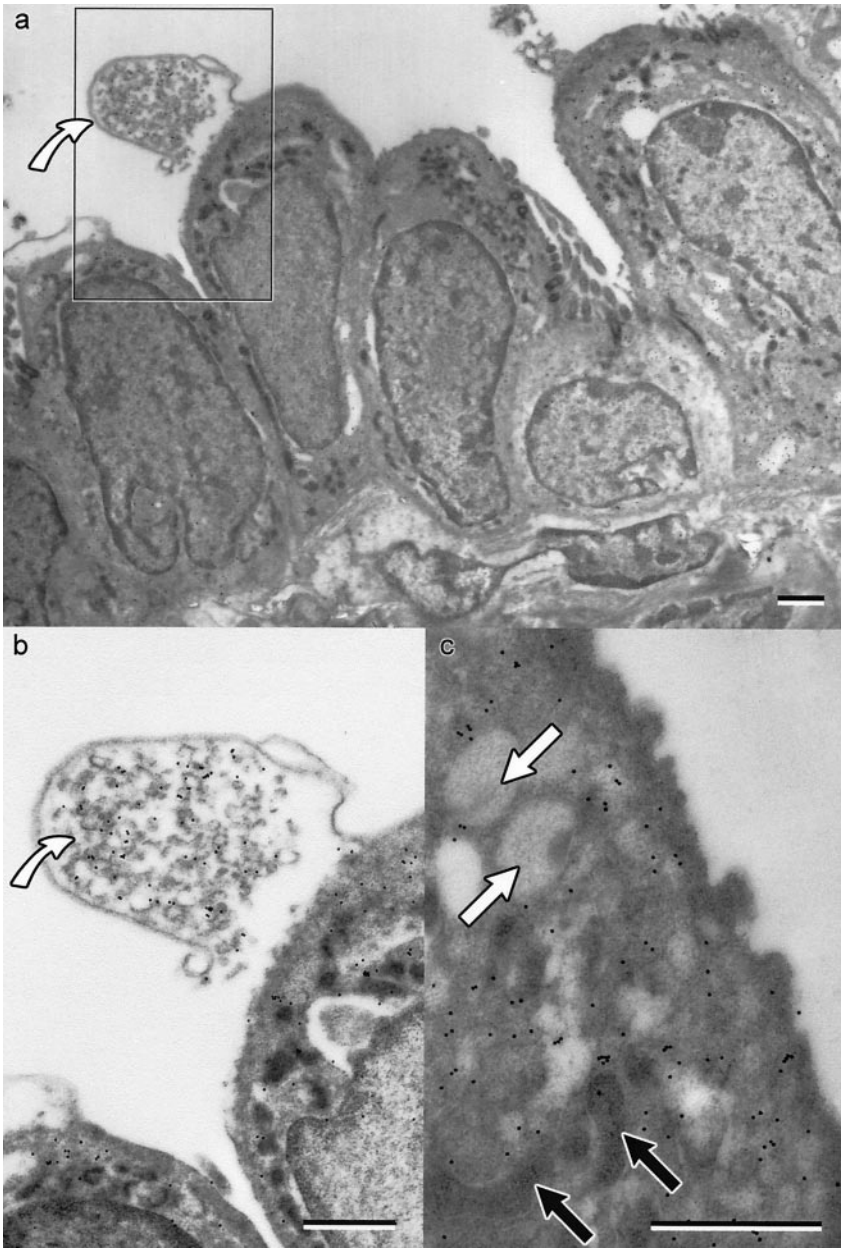


Fig. 5. Immunohistochemical localisation of CYP2B1 in bronchiolar tissue. (**a** and **b**) Clara cells, 24 hr after treatment with TMPT1 (50 mg/kg), show apocrine release of an apical "bleb" (curved white arrow) which is labelled with immunogold. (**c**) Clara cell from an untreated rat shows cytoplasmic immunogold labelling which is largely absent from secretory granules (white arrows) or mitochondria (black arrows), [all bars = 1 μm].

recent cell division were particularly common in the alveolar epithelium at this time (Fig. 4d). These cells were usually paired, their cellular and nuclear profiles were largely devoid of indentations, and most exhibited a particularly low incidence of immunogold label (0.9 ± 0.6 grains/μm^2, N = 12). In sections through the bronchioles, immunogold labelling was largely restricted to the cytoplasm of Clara cells from both treated (Fig. 5a) and untreated (Fig. 5c) rats. High concentrations of immunogold were evident over apical protrusions from these cells, which often showed signs of disruption within 24 hr of treatment with TMPT1 (Fig. 5a and b). Apical protrusions were less common 72 hr after treatment with TMPT1. The reduction in

Table 1
Effect of TMPT1 on the CYP2B1 content of type II and Clara cells

Treatment	Type II cells	Clara cells
Control	2.1 ± 1.1	11.8 ± 2.2
TMPT1 (24 hr)	1.9 ± 1.1	4.5 ± 1.4[a]
TMPT1 (72 hr)	1.7 ± 1.0[a]	3.0 ± 1.4[a]

Density of immunogold label (grains/μm^2) overlying the cytoplasm of type II and Clara cells following the treatment of rats with TMPT1 (50 mg/kg). All data have been normalised with respect to the level of nuclear labelling and are shown ± SD.

[a] Values significantly different from controls.

labelling over Clara cells, following dosing with TMPT1, was greater than that detected over Type II cells (Table 1). From a preliminary statistical analysis, a plot of fits and residuals showed that the latter were not normally distributed, and final analyses were thus conducted after a log transformation. The split-plot analysis of variance indicated that there was no significant variation within any treatment, including controls, for type II cell ($P = 0.473$) or Clara cell ($P = 0.158$) data. The reduction in immunolabelling following TMPT1 treatment was, however, significant for both type II ($P = < 0.001$) and Clara cells ($P = 0.017$). Tukey's test showed a significant difference between controls and all toxin-treated samples taken 72 hr after dosing. Analysis of data from sections incubated with non-immune serum did not detect any significant difference between the three groups of rats ($P = 0.81$).

4. Discussion

This study shows that injury to type I cells by TMPT1 caused a fall in the content of pulmonary CYP2B1 and similar injury, by the closely-related pneumotoxin TMPT2, caused a similar fall in the catalytic activity of this enzyme. In contrast, an equivalent inhibition of this activity, by a non-pneumotoxic analogue of these toxins, only resulted in a slight breakdown of this enzyme, and the loss of activity was immediately followed by the onset of a slow recovery.

Studies of pulmonary bioactivation have tended to concentrate on the Clara and type II cells, which comprise a much smaller component of lung tissue than type I cells, but have large profiles which are easily recognised by light microscopy. Unlike Clara and type II cells, type I pneumocytes cannot be isolated for culture and experimentation *in vitro*, but ultrastructural immunocytochemistry enables a simultaneous comparison of enzyme concentration in all these cell types. The presence of only one band, of the appropriate molecular weight, on immunoblots confirmed that the antibody used in the present study was detecting only one isoenzyme, i.e. CYP2B1, in rat lung [18,19]. Eukaryotic CYP enzymes are all membrane-bound and largely associated with the cytosolic face of the endoplasmic reticulum [20]. The nucleoplasm, rather than the surrounding resin, was thus chosen as the most appropriate region for the determination of non-specific background labelling. Other studies have shown CYP2B1 to be concentrated, as demonstrated in the present investigation, in the type I, type II, and Clara cells of rat lung [6,21,22]. These three cell types clearly account for most of the CYP2B1 protein within rat lung, but the relative importance of each cell type is difficult to determine.

Morphometric studies indicate that alveolar tissue constitutes 87% of the total lung volume in the rat [23], to which type I cells contribute more than twice the cellular volume of type II cells [24,25]. Thus, although the labelling intensity observed over type I cells in the present study was rather lower than that over type II and Clara cells, type I cells probably contain most of the pulmonary complement of CYP2B1. This conclusion is reinforced by reports that type II cells account for only about 10–15% [26] and Clara cells <5% [27] of all lung cells.

The presence of a high proportion of pulmonary CYP2B1 within type I cells is also consistent with the fall in both the concentration and the activity of this protein, in pulmonary microsomes, following the selective destruction of these cells by both TMPT1 and TMPT2. Butylated hydroxytoluene, which produces similar lesions in mice, also caused a dramatic fall in pulmonary microsomal CYP2B1, to 41% of control levels, 24 hr after dosing.[1] Reductions in various pulmonary monooxygenase activities have previously been reported following TMPT1 in rats [28] and butylated hydroxytoluene in mice [29], but the cells involved were not identified.

Despite the major impact of the deletion of type I cells, the changes in Clara and type II cells could exacerbate the loss of CYP2B1. The apocrine secretion of CYP2B1-rich smooth endoplasmic reticulum, observed in several Clara cells, may well affect the intensity of immunolabelling over the remaining cell, as well as contributing to the biotransformation potential of airway fluid [30]. A continued bioactivation is unlikely to account for the prolonged depletion of CYP2B1, as the half-life of trialkylphosphorothioates, in rats, is less than 1 hr and the effective dose reaching the lung after 24 hr is thus probably inconsequential [31]. It is conceivable that the slight fall in immunolabelling over type II and Clara cells, 24 hr after dosing, could result from destruction of the epitope by reactive metabolites formed within the smooth endoplasmic reticulum of these cells. Regeneration of the apoprotein probably takes much longer than the half-life of circulating trialkylphosphorothioates and thus could account for the slow recovery of CYP2B1. Another possible factor in the fall in immunolabelling of both microsomal blots and tissue sections is the onset of tissue repair involving increased numbers of recently divided Clara and type II cells that exhibited low levels of immunolabelling. The proliferation of type II cells is a common response to alveolar injury [32], and an increased division of Clara cells, even in the absence of bronchiolar injury, has been shown to be a very sensitive indicator of sublethal lung injury [33]. The decreased intensity of labelling over dividing type II and Clara cells could not account for much of the observed falls in pulmonary CYP2B, particularly as the area of these cell profiles increased prior to mitosis, but this process may exacerbate the losses caused by the destruction of type I cells. Significant levels of both the CYP2B1 apoenzyme and mRNA have been reported in freshly isolated type II cells, but these levels decline as the cultures differentiate [34]. This observation is consistent with previous studies on the progeny of dividing lung cells [35].

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In order to distinguish any possible effects caused by a loss of CYP2B1 activity from those of cellular injury, the lungs of rats dosed with TMPT1 were compared with those from rats dosed with the non-pneumotoxic analogue. Despite the dramatic fall in pulmonary CYP2B1 activity, no morphological correlates could be detected in any lung cells, but the decline in CYP2B1 activity was clearly accompanied by limited breakdown of the enzyme. The redistribution of label on immunoblots to a secondary band, within 2 hr of dosing, accounted for most of the immunolabelling that was lost from the primary band, and thus there was only a minimal reduction in total pulmonary CYP2B1 protein. The absence of any detectable change in the intensity of immunohistochemical labelling over type II and Clara cells is consistent with the breakdown product being retained within these cells. Redistribution of labelling within these cells could not, however, be detected.

In conclusion, the results of this investigation are consistent with the premise that a large proportion of pulmonary CYP2B1 is concentrated within the type I cells of the alveolar epithelium. Inhibition of this activity does not affect the morphology or immunolabelling of any type of lung cell. Selective destruction of type I pneumocytes results in a marked loss of the enzyme, which may be exacerbated and prolonged by the formation of numerous CYP2B1-deficient progeny of the apparently undamaged type II and Clara cells.

Acknowledgements

We are grateful to Ray Gilbert for the preparation of samples for electron microscopy/quantitation of immunogold labelling, and to Michael Festing for the statistical analysis of the data.

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