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Cytotoxicity of Bacterial-Derived Toxins to Immortal Lung Epithelial and Macrophage Cells

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Abstract Health risks associated with inhalation and deposition of biological materials have been a topic of great concern due to highly publicized cases of inhalation anthrax, of new regulations on the release of particulate matter, and to increased concerns on the hazards of indoor air pollution. Here, we present an evaluation of the sensitivity of two immortal cell lines (A549, human lung carcinoma epithelia) and NR8383 (rat alveolar macrophages) to a variety of bacterial-derived inhalation hazards and simulants including etoposide, gliotoxin, streptolysin O, and warfarin. The cell response is evaluated through quantification of changes in mitochondrial succinate dehydrogenase activity, release of lactate dehydrogenase, initiation of apoptosis, and through changes in morphology as determined by visible light microscopy and scanning electron microscopy. These cells display dose–response relations to each toxin, except for triton which has a step change response. The first observable responses of the epithelial cells to these compounds are changes in metabolism for one toxin (warfarin) and alterations in membrane permeability for another (gliotoxin). The other four toxins display a similar time course in response as gauged by changes in metabolism and loss of membrane integrity. Macrophages are more sensitive to most toxins; however, they display a lower level of stability. This information can be used in the design of cell-based sensors responding to these and similar hazards.

Keywords Human lung cells · Cell culture · Toxicity monitoring

Introduction

Public concern for the risks associated with inhalation of biohazardous materials has been raised due to several highly publicized cases of presumed exposure to anthrax, combustion wastes, and indoor air pollution. Health and safety risks of inhaling such materials are typically evaluated through extensive laboratory study. When the identity and composition

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of the potential toxin are unknown, this often requires extensive and expensive analysis using numerous laboratory animals. Such methods cannot provide suitable monitoring of the health impact of transient factors. The lack of a rapid means to directly quantify the health impact of inhalation hazards has led to the development of environmental regulations based on easily quantified metrics, such as the mass of particulate matter, which do not directly correlate to health risks. Current methods of detecting hazardous materials through traditional analytical procedures are lacking. New methods are required that can simultaneously detect multiple hazards (ranging across disparate chemical and biological classes) that can characterize functionality of the hazard and that can predict impact on humans and animals that may be contacted [1].

To address the need for a means to monitor the potential impact of inhaling air of dubious quality, cell-based biosensing devices are being developed, utilizing cell cultures that are responsive to many common inhalation hazards. These portable devices contain living biological cells that respond through physiological changes induced by exposure to environmental hazards including toxicants, pathogen, or other agents [1]. Such an approach can evaluate the potential toxicity of compounds relatively quickly, outside of the laboratory environment, inexpensively, and without the need for highly trained technicians. These biosensors are composed of two primary elements: a cellular layer which serves as a biological recognition element and a photometric sensor that quantifies the response of the cells to potential toxins.

Methods for detecting cell response include optical, electrical, and chemical means. Many of the recent works have focused on quantifying electrical responses of neurons or myocytes to biochemical agents. One group [2, 3] has used an integrated silicon-polydimethylesiloxane cell cartridge to monitor the action potential of cardiomyocytes exposed to biochemical agents. Thach and coworkers [4] found that both neural precursor cells and peripheral blood mononuclear cells were responsive for detection of Sindbis virus.

In the present study, we report on the response of human A549 lung carcinoma epithelial cells and rat alveolar macrophage cells to a wide range of chemicals that mimic the effects of biological agents. This response is quantified through standard biochemical analyses. Inhalation hazards evaluated include the following. Gliotoxin is a secondary metabolite produced from *Aspergillus* and *Penicillium* species and is present in agricultural dusts from plant surfaces, stored hay and grain, and compost with spores often in the respirable range. Acute exposures to gliotoxin are associated with respiratory allergic symptoms, while chronic exposures have been linked to aspergillosis, an immunosuppresion disease characterized by cough, fever, and shortness of breath [5, 6]. Methyl methansesulfonate (MMS) is an alkylating agent that causes single- and double-strand DNA breaks and developmental toxicity following exposure [7]. Streptolysin O is a thiol-activated cytolysin isolated from *Streptococcus pyogenes* which binds to cholesterol on the cell surface, permeabilizes cell membranes, and causes apoptosis [8]. Etoposide (VG-16) is a topoisomerase II inhibitor that binds to and cleaves DNA causing cross-linked DNA fragments. It is highly toxic to actively replicating cells and so is used as a chemotherapeutic agent to treat certain types of cancers such as leukemia. Triton X-100 is a detergent and has been used as a cytotoxic agent to induce cell death through extragenomic mechanisms [9]. Detergents affect the cell membrane by solubilizing the lipids through the disruption of hydrophobic contact, thus destroying the lipid bilayer. Warfarin (3-(a-acetonylbenzyl)-4-hydroxycoumarin) is an anticoagulant rodenticide used for controlling rats and house mice in homes, agricultural facilities, and industrial sites. Absorption by the lungs may result in hemorrhagic effects.

Materials and Methods

Cell Culture

A human lung carcinoma epithelia (A549) cell line and a rat alveolar macrophage (NR8383) line were maintained in Dulbecco's modified Eagle's media (Sigma Chemical Co., St. Louis, MO, USA), supplemented with 10% fetal bovine serum and 1% antibiotic–antimycotic solution (Sigma) in T-25 flasks at 37 °C and 5% CO_2. Relatively young cell cultures (passages less than 25) were used to ensure a consistent response [10, 11].

For A549 cytotoxicity experiments, newly confluent cell layers were trypsinized, resuspended in medium, and seeded in 96-well plates with 100 μL/well. Plates were seeded with 12,500 cells per well, which corresponds to 25% confluence over each well in the plate, and then allowed to grow to confluence for 48 h at 37 °C and 5% CO_2.

For NR8383 cytotoxicity experiments, a variation on procedures was necessary since these cells do not form strong attachments to surfaces. Cells were harvested, resuspended in fresh medium, and seeded in 96-well plates with 100 μL/well. Plates were seeded with 12,500 cells per well and then allowed to replicate for 48 h at 37 °C and 5% CO_2. After 48 h of growth, the number of cells per well was approximately 50,000.

All exposures were performed in serum-free medium as serum can interfere with dosages [12, 13]. Two columns of the 96-well plate (16 wells) were devoted to a control. Pure phosphate-buffered saline (PBS) solution was used instead of the toxic stock solution and were otherwise treated identical to the other wells.

Toxins

Gliotoxin is a secondary metabolite produced from *Apergillus* and was used to simulate a fungal exposure. Streptolysin O is a thiol-activated cytolysin isolated from *S. pyogenes* that permeabilizes cell membranes and causes DNA fragmentation. Streptolysin O binds to cholesterol on the surface of the cell membrane; multiple binding events lead to aggregation on the cell surface leading to formation of small pores on the cell membrane leading to cell swelling. Etoposide and methyl methansesulfonate are not naturally found in the environment but were used to simulate an exposure to genotoxic agents. Triton X-100 is a detergent and was used to simulate a cytotoxic agent. Warfarin (3-(a-acetonylbenzyl)-4-hydroxycoumarin) is an anticoagulant rodenticide used for controlling rats and mice in homes, agricultural facilities, and in industrial sites. Unless noted otherwise, all reagents were obtained from Sigma Chemical Co., St. Louis, MO.

Formulations of each toxin were prepared fresh before each experiment. MMS and triton were prepared in PBS at ten times the concentration to be evaluated on cultures. Etoposide was dissolved in a 2% dimethylsulfoxide (DMSO) solution; gliotoxin was dissolved in a 95% ethanol solution; streptolysin O was prepared in a phosphate buffer with dichlorodiphenyltrichloroethane [14]; and warfarin was dissolved in 5% acetone. These alternate solutions were developed to permit sufficient solubility of each toxin to reach concentrations beyond the TC_{50} value for each. The impact of DMSO, acetone, ethanol, and the streptolysin buffer were evaluated at concentrations matching those employed for exposures; typically, a 1:10 dilution is made with culture media for the exposures. No significant toxic effects were obtained (data not shown) at these exposure concentrations.

Cytotoxicity Quantification

Cells were exposed for various times ranging from 2 to 72 h before the response was quantified. Cellular metabolism was evaluated using an 3(4,5-dimethylthiazol-2-yl) 2,5-diphenyltetrazolium bromide (MTT) assay which used 5 mg/mL reagent in PBS prepared and filtered to remove undissolved solids immediately before each use. Ten microliters of MTT solution was added to the 100 μL of medium in each well, and the plate was incubated at 37 °C for 2 h. Absorbance was measured at 540 nm on a multiwell plate reader. Each measurement was performed with at least eight replicates for each data point; curves represent composites of two to three separate experiments.

NR8383 macrophage cell analysis was performed using a WST-1 assay (Roche Diagnostics GmbH, Mannheim Germany) following manufacturer's instructions and presented in greater detail previously [13, 15].

Cell death was quantified using a commercial nonradioactive lactate dehydrogenase (LDH) release assay (Roche Diagnostics GmbH, Mannheim Germany); manufacturer's procedures were followed. LDH release is an indicator of damage to plasma membrane integrity. Cell health was evaluated as a ratio of LDH release in response to toxins compared to that from lysed cells.

Mechanisms of cell death were investigated using a fluorescent-based assay (Oncogene Research Products, Boston, MA, USA) utilizing two fluorophores, annexin V-flourescein isothiocyanate (FITC) and propidium iodide. Annexin V-FITC identifies early apoptotic cells by binding to phosphatidyl serine present on the outer cell membrane only during early stages of apoptosis. Propidium iodide stains the DNA of cells with damaged membranes and identifies all dead cells. These fluorophores can differentiate between oncotic (nongene directed) and apoptotic (gene directed) cell death. The fluorescence-based assay was performed on confluent monolayers of A549 cells attached to polystyrene surfaces of a 12-well tissue culture treated plate (VWR) and exposed to toxins for 6–16 h. Cell counts of controls and exposed cells were tabulated and compared relative to the brightfield image (no fluorescence), which was used to determine total cell numbers in a microscopy field. Ten random fields were counted for each sample.

Scanning electron microscopy (SEM) images were collected on healthy and exposed A549 cells. Cells were grown to confluence on glass slides placed within a petri dish for 48 h after seeding. Cultures were exposed to toxins for 24 h and then prepared for SEM following standard procedures as described previously [13]. A549 cells were seeded onto 2.5×2.5 cm cover slips at a cell density of 25,000 cells per cover slip. Concentrations of etoposide, MMS, and triton above and below the TC_{50} values were selected and cells were exposed for 24 h following procedures described above. TC_{50} was defined as the concentration of toxicant causing a decrease in cellular function by 50% as gauged by metabolic assay. Images were viewed under the SEM using an accelerating voltage of 12 kV, condenser lens at 1–3 o'clock, and working distance ranging from 20 to 24 mm. All images were saved with a resolution of at least 640×640 pixels.

The presence of statistical differences between groups was determined using analysis of variance, with Newman–Keuls method as the post hoc test [16]. Each value presented in figures below represents the mean±standard deviation for between eight and 16 experimental treatments. The level of statistical significance employed was either $P<0.05$ or $P<0.01$.

Results

The sensitivity of transformed lung cell cultures to a variety of toxins was evaluated through analysis of morphology (SEM), metabolic activity (MTT), membrane integrity

Table 1 Summary of TC_{50} values for 24 h exposure and gene expression changes in response to compounds tested here (Boesewetter et al. [7]).

Toxin	Predominant alterations in gene expression (Boesewetter et al. [7])	TC_{50} value for A549	TC_{50} value for NR8383
Etoposide	Cell cycle, DNA repair enzymes, electron transport, and apoptotic pathways	1.0 mM	0.06 mM
Gliotoxin	Metabolic enzymes, ion channels, signaling pathways, cell adhesion	0.0005 mM	0.00003 mM
MMS	Cell cycle, protein translation, DNA repair enzymes, and apoptotic pathways, immune response cascades	1.1 mM	0.75 mM
Streptolysin O	Ion channel proteins, structural proteins, signal transduction, protein modification, and metabolic enzymes	680 units	75 units
Triton X-100	Extracellular matrix, ion channel proteins, structural proteins, cell cycle, and metabolic enzymes	0.09 mM	0.05 mM

(LDH release and propidium iodide uptake), and initiation of apoptosis (annexin V assay). Cell health is presented as a ratio the function of exposed cells to that of unexposed controls.

The behavior of A549 cells and NR8383 cells to varying concentrations of toxins for 24 h shows a clear dose response. For both cells, gliotoxin generates a response at the lowest concentrations (Table 1). Shown here are the results for streptolysin O only (Fig. 1); similar behavior is seen for response to the other toxins tested. In general, NR8383 macrophages are more sensitive to toxins and generate a response at lower concentrations. Note, however, that these macrophage responses display greater variability in sensitivity and in several cases demonstrate a step change response rather than a smooth decline compared with the more predictable response of the A549 epithelial cells. Temporal studies were performed to gain insight into mechanisms of cellular response.

The temporal response of A549 cells to varying concentrations of each toxin is shown in Fig. 2. Etoposide (Fig. 2a) caused little decline in cellular metabolic activity after 2 h of exposure. A dose response appeared after 8 h of exposure. Metabolic activity continued to

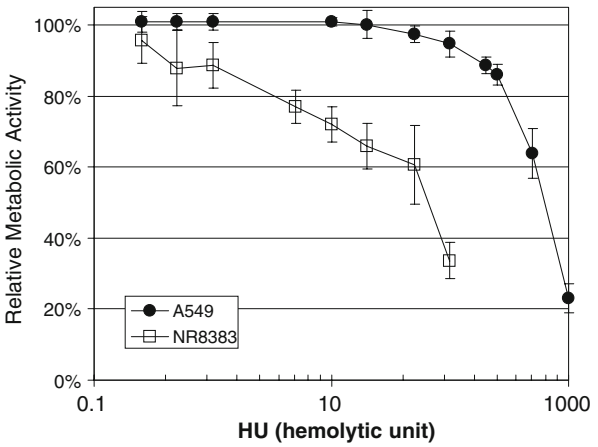

Fig. 1 Response of A549 and NR8383 cells to toxins quantified as metabolic activity after 24 h of exposure to streptolysin O

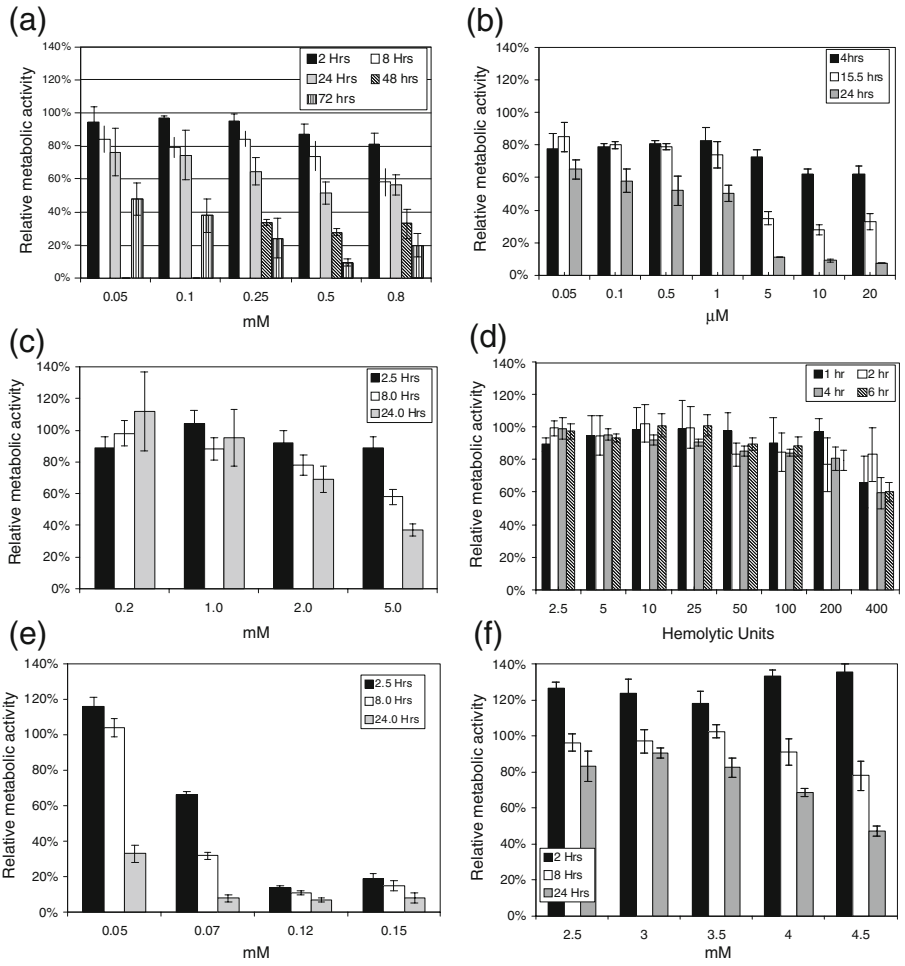

Fig. 2 Temporal response of A549 cell metabolic activity after exposure to: **a** etoposide; **b** gliotoxin; **c** MMS; **d** streptolysin O; **e** triton; **f** warfarin

decline past 48 h of exposure. The temporal behavior of declined metabolism is supported by LDH release measurements (Fig. 3a) in response to etoposide. However, nearly the same amounts of LDH were quantified after low and high etoposide exposures.

Exposure of the A549 cells to either 0.01 or 0.1 mM etoposide for 16 h led to a significant increase in the number of cells in the early stages of apoptosis (Fig. 4a). Very few cells were stained by propidium iodide at each concentration suggesting that etoposide caused minimal loss of membrane integrity. At 0.01 mM, approximately 16% of the cells were shown to be exhibiting early stages of apoptotic mechanisms compared to almost 30% after exposure to 0.1 mM etoposide.

Upon exposure to etoposide, the A549 cells underwent a moderately rapid morphological change as displayed in Fig. 5. When exposed to concentrations of 0.5 mM for 30 min (not shown), cell morphology changed little compared to that of unexposed controls (Fig. 5a); however, after exposure for 14 to 24 h (Fig. 5d, e, respectively) the cells showed a sizeable change in morphology with most cells highly rounded and condensed (Fig. 5f).

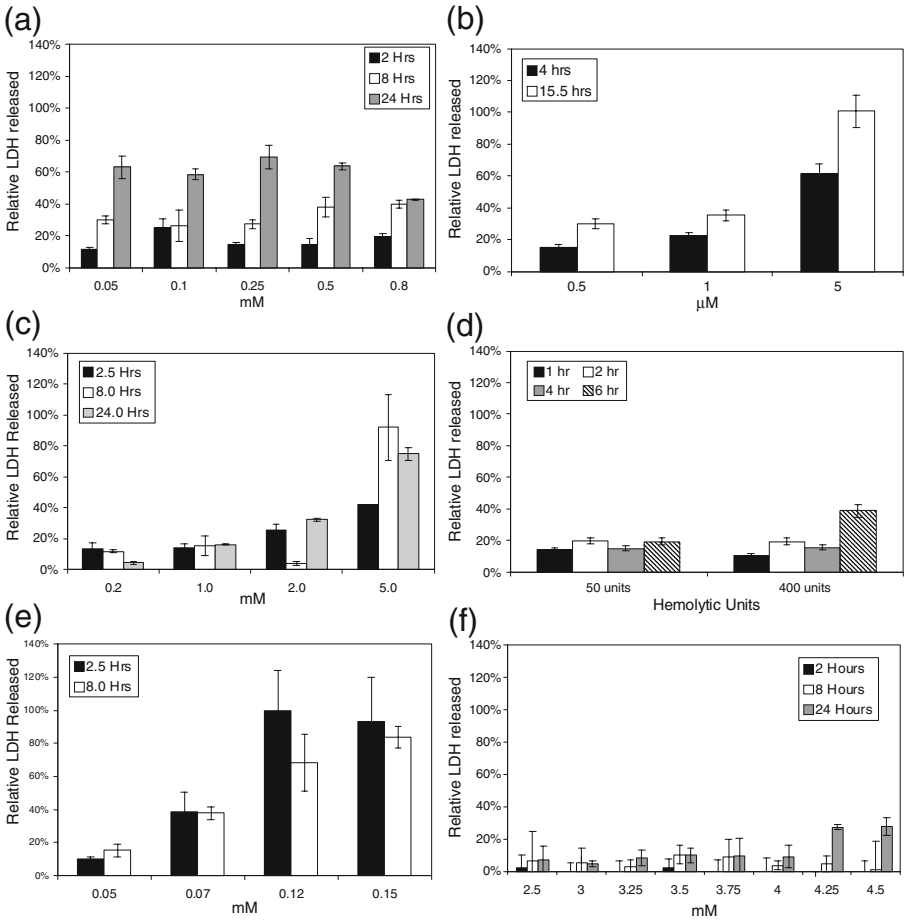

Fig. 3 Temporal response of A549 cell LDH release in response to: **a** etoposide; **b** gliotoxin; **c** MMS; **d** streptolysin O; **e** triton; **f** warfarin. Each measurement was performed with at least eight replicates for each data point

Fewer cells were present on the surface after 24 h (Fig. 5e) than at 14 h (Fig. 5d); all cultures began with a confluent monolayer.

Gliotoxin (Fig. 2b, Table 1) elicited decreases in metabolic activity of at least 20% for all exposure concentrations within 4 h of exposure. Above a concentration of 0.001 mM, a large decrease was apparent after 15 h, but activity was still on the order of 40%. Only after 24 h of exposure, did metabolism decline to very low levels. This long time to respond is not fully supported by LDH release measurements (Fig. 3b) which showed that nearly 70% of the available LDH was released within 4 h of exposure to high gliotoxin concentrations.

A549 cells exposed to 0.005 mM gliotoxin for 6 h displayed only a small portion of cells labeled with the annexin V-FITC, indicative of early apoptosis (Fig. 4b). An even smaller proportion displayed loss of membrane integrity. This supports the MTT results but not the LDH release measurements.

Exposure to MMS yielded minimal effect after 2 h but significant decline in metabolic activity (Fig. 2c, Table 1) and LDH release (Fig. 3c) after 8 h for the highest concentrations.

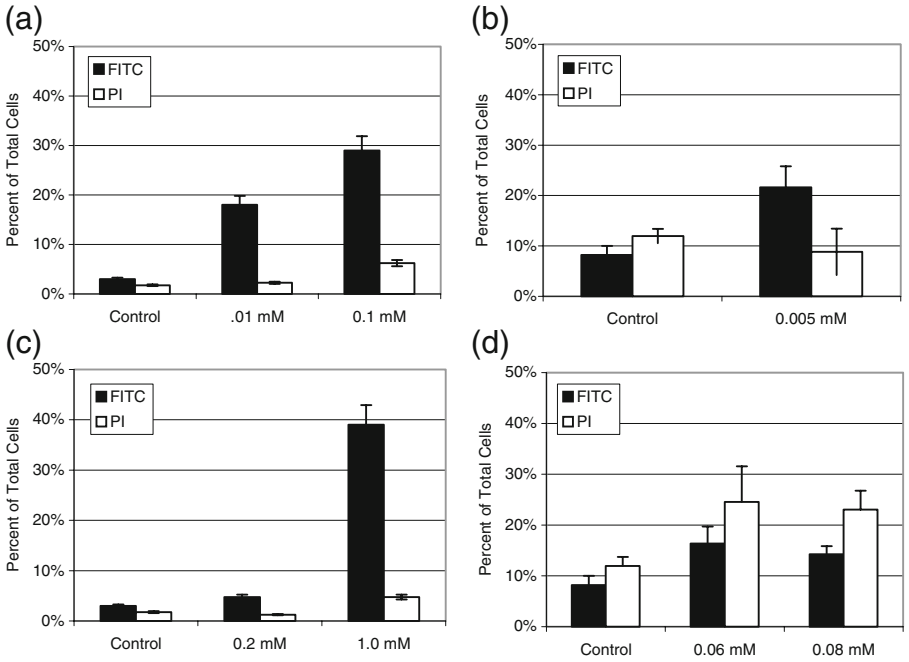

Fig. 4 Response of A549 cells as gauged by annexin V-FITC (early apoptosis) and propidium iodide (*PI*) for loss of membrane integrity. **a** Etoposide; **b** gliotoxin; **c** triton; **d** MMS. Etoposide and MMS were evaluated after 16 h, while gliotoxin and triton were evaluated after 6 h

Minimal LDH can be quantified after exposure to low concentrations of MMS. The differences in metabolic activity were more pronounced at the higher exposure concentrations. A significant number of A549 cells exposed to 1.0 mM MMS for 16 h displayed evidence of early apoptosis (Fig. 4c). After 24 h of exposure, cell morphology shows significant "blebbing" with a loss of cell–cell contact (Fig. 5g–i).

Exposure of A549 cells to streptolysin O concentrations below the TC_{50} of 680 units yielded a slow decline in cell viability. Culture metabolism (Fig. 2d, Table 1) remained close to that of unexposed controls, except for those exposed to 400 units. Despite having the membrane as the primary target for attack, streptolysin O induced little LDH release up to 6 h of exposure (Fig. 3d).

Triton X-100 caused a very rapid decline in culture metabolic activity for concentrations above the TC_{50} level (Fig. 2e, Table 1). Essentially no metabolic activity was apparent after exposure to greater than 0.1 mM triton for 2.5 h or more. This was supported by the release of nearly all of the available LDH (Fig. 3e). Lower concentrations of triton did promote significant declines in metabolic activity (Fig. 2e), but these declines were apparent only after longer times of exposure.

A549 cells exposed to 0.06 and 0.08 mM Triton for 6 h (Fig. 4d) showed minimal annexin V-FITC staining compared to the unexposed control; however, a larger proportion of cells stained with propidium iodide. At both these concentrations, metabolic activity should have declined to 40% that of controls (Fig. 2e). When exposed to low

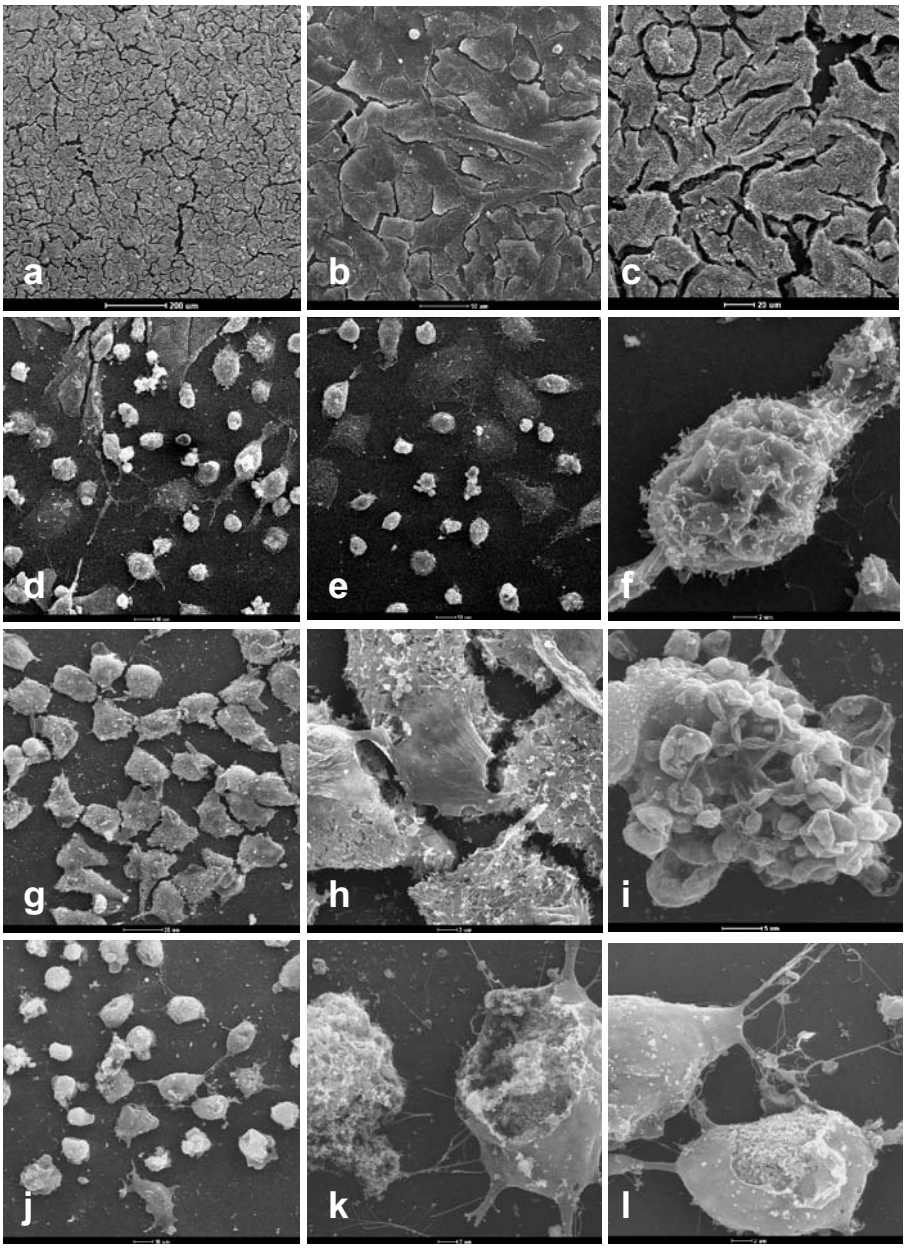

Fig. 5 SEM of A549 cells exposed to biotoxins. **a** control (×80); **b** control (×250); **c** control (×400); **d** etoposide, 14 h, (×500); **e** etoposide, 24 h, (×500); **f** etoposide, 14 h, (×3,000); **g** MMS, 24 h (×500); **h** MMS, 24 h (×2,500); **i** MMS, 24 h (×2,500); **j** triton, 24 h (×500); **k** triton, 24 h (×2,500); **l** triton, 24 h (×2,500)

concentrations of Triton for up to 60 min (not shown), cell morphology changes little compared to that of unexposed controls (Fig. 5a). After exposure to 0.1 mM Triton for 24 h (Fig. 5j–l), the cells lose their tight packing and show gross mechanical damage evidenced primarily by an apparent loss of large areas of the cell membrane.

Warfarin acts slowly on A549 cells with minimal impact on metabolism seen after 2 h of exposure; however, a dose response appears after 24 h of exposure (Fig. 2f). This compound has minimal impact on the cell membrane as evidenced by the very low amount of LDH released regardless of time or exposure concentration (Fig. 3f).

Discussion

These analyses provide information on the sensitivity of immortal cells (A549 human type II epithelia and rat alveolar macrophages) and on mechanisms and timing of damage resulting from exposure to a variety of biochemical toxins. The biochemicals were selected based on their ability to induce different types of cellular damage and hence induce different cell response mechanisms. Cytotoxicity can be cell-type dependent [17], and so all toxins were matched here with either cellular barriers or potential targets. A549 cells are commonly applied as a cell culture tool for assessing potential inhalation toxicity [18]. The metabolism of this epithelial layer is often one of the first cellular functions altered by the presence of toxins [19].

In A549 cells, etoposide has been reported to induce double-strand DNA breaks after 8 h while the cells remained viable [9]. Etoposide was shown to rapidly and profoundly upregulate p53, an inducer of apoptosis [20, 21], in the later phases of apoptosis but immediately before caspase-3 activation [21]. The result is that etoposide blocks the cell cycle in S-phase and G2-phase in normal and tumor cell lines.

Our results suggest there is a moderately quick cell decline through apoptosis in response to etoposide, which caused little decrease in cellular metabolic activity or LDH release after 2 h, but displayed a dose response after 8 h of exposure. Metabolic activity continued to decline through 48 h of exposure. Etoposide induced apoptosis in a dose-dependent manner, suggesting a strong genetic component to the response.

Gliotoxin is a fungal toxin that acts as an immunosuppressent, but its mechanism of action is largely unknown and varies based on the type of cell it encounters. A549 cells were shown to have a concentration-dependant growth inhibition to gliotoxin [22] which could be attributed to reactive oxygen species generated through redox cycling [23–25]. In rat macrophages and human colonic epithelial cells, gliotoxin inhibited NF-κB activation and significantly inhibited cytokine secretion when stimulated with lipopolysaccharide [26].

The response to gliotoxin seen here was fairly slow, with only small declines in cell function after 4 h of exposure; however, substantial LDH was released within this time frame. This supports the mechanism of membrane damage but does not support the altered metabolic flux pattern at short times. As shown here, gliotoxin could induce oncotic cell death but only a small degree of apoptosis, even at fairly high concentrations. A possible mechanism of cell response to gliotoxin is damage to membrane lipids followed by damage to intracellular proteins. The macrophages are significantly more sensitive to gliotoxin than are the A549 cells (TC_{50} 0.00003 mM for NR8383 and 0.0005 mM for A549). Of the compounds tested here, gliotoxin has the greatest difference in sensitivity between these two cell types. This could be due to the different abilities of these cells to generate oxygen free radicals (higher in the macrophages).

Methyl methansesulfonate is an alkylating agent that causes single- and double-stranded DNA breaks. This genotoxin was expected to induce similar responses in A549 cells as etoposide [27]. A549 cells exposed to MMS experienced a substantial increase in the number of apoptotic cells, and this exceeds the decline in mitochondrial activity or loss of membrane activity at this MMS concentration. Apparently, the early stage of apoptosis

initiated by MMS does not greatly impact mitochondrial succinate dehydrogenase activity or membrane integrity. The TC_{50} values for the A549 cells and the NR8383 are quite similar, suggesting a fairly ubiquitous alkylating damage.

Streptolysin O is a bacterial pore-forming agent involved in the pathogenesis of a number of gram-positive bacterial species. It binds to cholesterol on the surface of the cell membrane; multiple binding events lead to aggregation on the cell surface leading to formation of small pores on the cell membrane, leading to cell swelling.

Results with streptolysin O indicate that it is a potent inhibitor of cell metabolism but acts rather slowly. Impact on cell attachment is minimal, and LDH release lags behind metabolic inhibition. This is surprising, given the mechanism of action and pore formation, but suggests a relatively slow acting process in damaging the cell membrane. Macrophages are more sensitive than are epithelial cells with a TC_{50} nearly an order of magnitude lower than that for epithelial cells; this is likely due to the differing amount of cholesterol in these membranes. Macrophages have approximately 364 μg cholesterol/mg total cell protein [28], whereas A549 cells have approximately 15 μg cholesterol/mg protein [29]. The 20-fold higher cholesterol level of the macrophages could account for the 9-fold greater sensitivity of the macrophages to streptolysin O.

Triton destabilizes cell adhesion by solubilizing the cell membrane with complete solubilization occurring at concentrations of 2% *v*/*v*, corresponding to 32 mM (average MW=625 D), which is significantly higher than that evaluated here. The hydrophobic ends of the detergent bind to the hydrophobic regions of the proteins dislocating the lipid molecules.

The TC_{50} for A549 cells and NR8383 cells responding to triton is fairly similar (0.09 mM and 0.05 mM, respectively), suggesting some similarity in the type of damage that is caused in each of the cell types. The greater sensitivity of the macrophages could again be due to their higher cholesterol content which maintains higher membrane fluidity. Both the A549 and NR8383 cells show a step change response to triton within a narrow range of concentrations. Only a small amount of apoptosis was detected; however, triton exposure does lead to initiation of complex responses (Table 1 and [7]). Triton leads to substantial increases in the transcription of genes for structural and transport proteins. The effect of triton likely extends beyond just the membrane, and so its use as a membrane-permeabilizing agent should be utilized cautiously.

Warfarin is odorless, tasteless, and effective in very low dosages as a preventative of blood clotting. Its action is considered to not be rapid. Warfarin acts by inhibiting vitamin K-dependent coagulation factors and so is also utilized as a pharmaceutical to prevent blood from clotting to treat acute pulmonary embolism, atrial fibrillation, and deep vein thrombosis and after a heart attack or stroke to prevent a recurrence. Absorption by the lungs may result in hemorrhagic effects.

Our results with warfarin indicate that it impacts metabolic activity at lower concentrations than it impacts membrane integrity. It is a slow-acting agent on A549 cells producing a small increase in metabolism after 2 h exposure followed by a decline in mitochondrial activity occurring only after 24 h of exposure. Its low solubility may lead to the slow uptake into A549 cells.

The response of A549 and NR8383 lung-derived cells to these toxins is used as a biological recognition element in spectroscopic biosensors to detect the presence and identity of inhalation health hazards [30, 31]. As shown here, both cell types are highly responsive to a wide range of toxins including those that induce membrane damage, DNA damage, and inhibition of metabolic activity. We have found that membrane damage is readily quantified using a fiber-delivered infrared spectroscopic measurement [32–33],

whereas internal damage may best be quantified through Raman spectroscopic measurements [34]. Macrophages appear to be more sensitive to many of these biotoxins; however, their low adherence to surfaces and high variability in response makes their use in a biosensor problematic and requires further evaluation.

Conclusions

The A549 and NR8383 cells were sensitive to a wide range of biological toxins. Both cell types show some response to each toxin after 2 h of exposure (the shortest time tested) with the degree of response depending on the concentration of toxicant. The response to Triton X-100 was most rapid with nearly all metabolic activity lost in 2.5 h after exposure to amounts above the TC_{50}. This surfactant caused little damage at low concentrations but yielded a step change in decline of cell function around the TC_{50} value. No other toxicant showed such a rapid decline in cell function even at the highest concentrations tested. Response followed dose-dependent behavior with gradual declines in cell function for all compounds other than triton X-100. Gliotoxin was the most potent toxicant requiring only micromolar levels to inhibit cellular metabolism and showed a substantial interaction between dosage and exposure time with cellular response.

These studies support the use of A549 and similar cultures as biological recognition elements to detect inhalation health hazards. Judicious selection of cell types used in cell-based biosensors must be based on application, detection methodology, and environmental conditions to which the cells will be subjected. The higher sensitivity of the macrophages to these toxins suggests that they may be suitable for use as a biological recognition element; however, their low adhesion to surfaces makes their use problematic for this application.

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