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Short communication

The effect of *m*-xylene on cytotoxicity and cellular antioxidant status in rat dermal equivalents

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Abstract

Exposure of the skin to volatile organic chemicals (VOCs) can lead to irritation, inflammation and cytotoxicity. Since VOCs are used in industrial, commercial and military applications, concern is mounting with respect to VOC safe exposure limits. Although traditional toxicological assessment of VOCs has utilized animal models, the use of alternative in vitro models is becoming more widespread. We have previously developed a sealed exposure system that prevents chemical loss through evaporation and enables calculation of target cell chemical dose. The present study utilized this in vitro exposure method to assess *m*-xylene-induced cytotoxicity and antioxidant status in dermal equivalents (dermal fibroblasts in a collagen matrix). At the end of a 1- or 4-h exposure, cytotoxicity was measured using the MTT assay and the EC_{50} values determined were 1481 ± 88 and 930 ± 33, respectively. Decreases in cellular thiols and catalase activity were observed, which occurred in a time and dose-dependent manner. Treatment of dermal equivalents with the antioxidants *N*-acetylcysteine (NAC) and catalase provided some protection against *m*-xylene-induced cytotoxicity. When compared to *m*-xylene exposures, treatment with either 1.0 or 5.0 mM NAC led to increases in the EC_{50} values at 1 and 4 h. Increases in these EC_{50} values ranged from 1.22- to 1.32-fold at 1 h and 1.27- to 1.54-fold at 4 h. Although treatment with catalase (1000 U/ml) led to a 1.35-fold increase in cell viability at 1 h, no significant differences were observed at either 1 or 4 h when compared to dermal equivalents exposed to *m*-xylene alone. These results suggest that exposure to *m*-xylene leads to a time- and dose-dependent decrease in cellular antioxidants and that cellular thiols may provide protection against the cytotoxic properties of *m*-xylene.

Keywords: Skin; Irritation; *m*-Xylene; Dermal equivalent; *N*-acetylcysteine; Catalase

1. Introduction

Reactive oxygen species can be formed during skin inflammation and promote skin damage.

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Under normal physiological conditions, endogenous antioxidants such as glutathione, catalase, superoxide dismutase, glutathione peroxidase and other endogenous antioxidants protect the cell from oxidative damage. However, an abundance of free radicals can lead to the exhaustion of antioxidant protective mechanisms, which can render cells susceptible to lipid peroxidation and damage to DNA and proteins (Berlett and Stadtman, 1997; Cadet et al., 2001). Dermal application of irritants, such as sodium lauryl sulfate, dithranol, 1-chloro-2, 4-dinitrobenzene, anthralin, along with UV light exposure, promotes oxidative stress in the skin (Kimura et al., 1998; Lange et al., 1998; Katiyar et al., 1999; Willis et al., 2001). Topical treatment of the skin with antioxidants can reduce the severity of oxidative stress-induced dermal irritation (Dreher and Maibach, 2001).

Volatile organic chemicals (VOCs) are compounds used in industrial, commercial and military environments for the purposes of fuel production, paint thinners, metal degreasers, chemical synthesis intermediates and consumer products. Dermal uptake of VOCs in the form of vapor, liquid or aerosol primarily occurs through diffusion into the stratum corneum. Once VOCs have penetrated the stratum corneum, damage to the viable epidermis and dermis can result in erythema, edema, increased skin blood flow, leukocyte infiltration, necrosis and increased levels of oxidative species and low molecular-weight DNA (Steele and Wilhelm, 1966, 1970; McDougal et al., 1997; Rogers et al., 2001).

Chemicals are classified as irritants or non-irritants, although there is no reliable method to estimate the severity of irritation solely based on chemical structure, penetration rate or duration of exposure (de Brugerolle de Fraissinette et al., 1999). Throughout the last several decades, a standard method for determining chemical irritancy has been the Draize test (Draize et al., 1944). While in vivo tests have been used to approximate dermal exposures, the need for chemical irritancy testing has led to the increased development of alternative in vitro approaches (Augustin and Damour, 1995; de Brugerolle de Fraissinette et al., 1999; Perkins et al., 1999; Fentem et al., 2001). Correlative results were observed between in vitro and in vivo models when comparing chemical irritancy results using the same chemical and cell type (de Brugerolle de Fraissinette et al., 1999; Perkins et al., 1999; Lee et al., 2000). Many different in vitro models have been used to evaluate chemical-induced skin irritation, including normal and immortal keratinocyte and fibroblasts monolayers, skin explant cultures, skin equivalents or dermal equivalents (Augustin and Damour, 1995; de Brugerolle de Fraissinette et al., 1999; Perkins et al., 1999; Lee et al., 2000; Yang et al., 2000; Fentem et al., 2001). Currently, there is no single in vitro model that is used for irritation testing, however a pre-validation study using EpiDerm™, EPISKIN™ and PREDISKIN™ has been conducted to determine whether these in vitro models are appropriate for irritant testing (Fentem et al., 2001). A follow-up to this pre-validation study recommended further evaluation of these model systems (Zuang et al., 2002).

In vitro systems could be used for the development of predictive biologically-based mathematical models and other risk assessment purposes. However, a problem that occurs while conducting in vitro exposures with VOCs is chemical evaporation from the exposure medium. This evaporation can ultimately result in decreasing chemical concentration throughout the exposure period, potentially affecting specific endpoint values. Our laboratory has previously developed an exposure method for assessing the dermal toxicity of VOCs, which maintains a constant chemical dose throughout the exposure period and enables calculation of chemical dose in the cells, not just the exposure medium (Rogers and McDougal, 2002). Xylene is considered an irritant and we have previously observed *m*-xylene-induced oxidative species formation in whole skin (Rogers et al., 2001), therefore the purpose of this study was to determine whether oxidative stress plays a role in the cytotoxic effect of *m*-xylene in rat dermal fibroblasts. This study will aid in the evaluation of our previously developed exposure method for VOCs by monitoring the time- and dose-dependent relationships of specific toxicological endpoints.

2. Materials and methods

2.1. Cell culture

Male Fischer F-344 rat dermal fibroblasts were grown in Ham's F-12/DMEM without Phenol red (Sigma, St. Louis, MO), supplemented with 10% fetal bovine serum, 100 U/ml penicillin, and 100 μg/ml streptomycin at 37 °C under 95% air and 5% CO_2. Cultures maintained through ten passages were used for exposures. Dermal equivalents were constructed as previously described (Rogers and McDougal, 2002). Briefly, a solution containing medium, rat-tail collagen in 0.1% glacial acetic acid and NaOH (to adjust pH to 7.4) was mixed and maintained on ice. In each well of a 24-well plate, 500 μl of the collagen solution was combined with 2.5×10^5 rat dermal fibroblasts. This solution was mixed and immediately placed in an incubator at 37 °C under 95% air and 5% CO_2. The dermal matrices were allowed to congeal for a minimum of 1 h and 1.0 ml of fresh medium was added to each well. Dermal equivalents that contracted to $\approx$5–6 mm in diameter after 1–2 days in culture were used for *m*-xylene exposures.

2.2. Dermal equivalent exposures

Chemical exposures were conducted as previously described (Rogers and McDougal, 2002). Briefly, initial *m*-xylene dosing solutions were prepared by adding 5.0 μl *m*-xylene to sealed 12.0 ml Hewlett-Packard headspace vials completely filled with complete culture medium. No vehicle (e.g. ethanol) was used to prepare the dosing solutions. On the day of exposure, four dermal equivalents were added into each of six separate 2.0 ml glass exposure vials (Target I-D™ Vial; National Scientific Co., Lawrenceville, GA). Dilutions (1:10, 1:5, 1:2.5, 1:1, 0) of initial dosing solutions were prepared by combining fresh complete culture medium with the dosing solution in a gastight syringe. Each vial was filled with a separate dilution of exposure medium, immediately sealed with a Teflon-coated rubber septum and incubated at 37 °C for 1 or 4 h. Sham-exposed (medium only) samples were used as controls. At the end of the exposure, 1.0 ml of the exposure medium was removed from each vial using a gastight syringe and 500 μl was injected in duplicate into pre-weighed 21.5 ml Kimble headspace vials. The vials were incubated for 3 h at 80–85 °C on a vortex evaporator and a 0.1 ml sample of the headspace was analyzed using a Hewlett-Packard 5890 Series II Gas Chromatograph (Hewlett-Packard Co, Palo Alto, CA) containing a J&W Scientific DB-5 column (J&W Scientific Inc., Folsom, CA) and EZChrom Elite software (Scientific Software, Inc, Pleasanton, CA). The medium *m*-xylene concentration (determined from a standard curve) was expressed as μg/ml. The dermal equivalents were removed from each vial and assessed for cell viability or protein content and levels of cellular thiols and catalase activity.

2.3. Fibroblast m-xylene concentration

The *m*-xylene concentration in the fibroblasts of the dermal equivalents was determined as previously described using partition coefficients (Rogers and McDougal, 2002). The use of partition coefficients enables calculation of chemical concentration in the fibroblasts once the exposure medium chemical concentration is determined by GC analysis. The fibroblast:medium partition coefficient for *m*-xylene was previously found to be 17.9 (Rogers and McDougal, 2002), which means the cellular *m*-xylene concentration (μg *m*-xylene/g cells) is $\approx$17.9 times higher than the media *m*-xylene concentration (μg *m*-xylene/ml). The formula $C_c = (PC_{f/m})(C_m)$ calculates the cellular *m*-xylene concentration (C_c) as μg *m*-xylene/g cells using the fibroblast:medium partition coefficient for *m*-xylene ($PC_{f/m}$) and the exposure medium *m*-xylene concentration (C_m) as determined by GC analysis at the end of the 1- or 4-h exposure.

2.4. Cell viability

The viability of the fibroblasts in the dermal equivalents was determined by measuring the reduction of tetrazolium dye MTT in active mitochondria (Mosmann, 1983). Following chemical exposure, the dermal equivalents were incubated separately in 500 μl of 0.5 mg/ml MTT in

PBS (pH 7.4) for 1 h at 37 °C. Following incubation, each dermal equivalent was placed in a 1.5 ml microcentrifuge tube containing 700 μl of acidified isopropanol. Each sample was sonicated for 5–10 s and then centrifuged for 5 min at 12,000 × *g*. Aliquots (200 μl) of the isopropanol supernatant were added in triplicate to a 96-well plate and the absorbance was read at 570 and 630 nm using a SPECTRAMAX 190 microplate reader (Molecular Devices, Sunnyvale, CA). The difference in absorbance at 570 and 630 nm was calculated and the viability of the fibroblasts was expressed as percent to sham-exposed controls. The EC_{50} (effective *m*-xylene concentration at which 50% cell viability was observed) of the viability curves was determined from each of the four independent experiments using non-linear (sigmoid, three parameter) regression analysis (SigmaPlot, SPSS Science, Chicago, IL).

2.5. Endogenous catalase and thiols assays

The determination of protein levels was used for normalization between individual dermal equivalents. Following exposure, individual lattices were placed in separate wells of a 24-well plate, washed twice for 15 min in PBS (pH 7.4), placed in microcentrifuge tubes containing 500 μl of PBS, sonicated on ice for 5–10 s and centrifuged at 12,000 × *g* for 5 min. The supernatant was removed and the protein levels were determined using the BCA protein assay kit (Pierce, Rockford, IL) according to the manufacturer's instructions. The samples were read on a SPECTRAMAX 190 microplate reader at 562 nm and protein values were derived from a standard curve. Remaining supernatant samples were assayed for catalase activity and thiols.

Endogenous catalase was measured as previously described (Moysan et al., 1993) with slight modification. A reaction mixture was prepared consisting of 30 mM H_2O_2 in potassium phosphate buffer (0.05 M; pH 7.0). Ten microliters of the sample supernatant was added in triplicate to a 96-well plate, 200 μl of the reaction mixture was added to each sample. The absorbance in each well was read at 0, 5, 10, 15 and 30 min on a SPECTRAMAX 190 microplate reader (Molecular Devices) using a wavelength of 240 nm. Catalase activity was determined as the amount of H_2O_2 consumed per minute (Moysan et al., 1993). Values were normalized to protein and expressed as a percent of sham-exposed controls.

Endogenous thiols in the dermal equivalents were detected using Ellman's Reagent, also known as DTNB (5,5-dithiobis (2-nitrobenzoic acid)), which reacts with sulfhydryl groups in tissues and proteins (Gergel and Cederbaum, 1997). Samples (100 μl) of the cellular preps from the dermal equivalents were placed on a 96-well plate in triplicate, 200 μl of DTNB was added to each well, the absorbance was immediately read at 412 nm. Data were normalized to protein and expressed as a percent of sham-exposed controls.

2.6. NAC and catalase pre-treatment

To determine whether antioxidants may protect against *m*-xylene-induced cytotoxicity, dermal equivalents were pretreated with *N*-acetylcysteine (NAC) or catalase prior to *m*-xylene exposure. The dermal equivalents were incubated with 1.0 or 5.0 mM NAC (Sigma) or 1000 U/ml catalase (Roche, Indianapolis, IN) for 16–18 h. Following antioxidant treatment, dermal equivalents were exposed to *m*-xylene for 1 or 4 h and assessed for viability as described above. Catalase (1000 U/ml) was also present during exposure to *m*-xylene.

To determine whether the concentrations of NAC or catalase used were toxic to the fibroblasts, additional exposures were conducted using only NAC or catalase. Dermal equivalents were exposed to 1.0 or 5.0 mM NAC or 1000 U/ml catalase for 18 h. At the end of exposure, the tissues were assessed for viability using the MTT assay and compared to sham-exposed (medium only) controls.

2.7. Statistical analysis

Data were expressed as mean ± S.E.M. The *t*-test, one-way ANOVA and multiple regression analysis were used for the data analysis (Statistica, StatSoft, Tulsa, OK) The *t*-test compared the difference in EC_{50} values from chemical exposed and NAC or catalase pre-treated samples. The

one-way ANOVA and multiple regression analysis (Statistica) were used to compare the changes in catalase or thiol levels at 1 and 4 h. $P < 0.05$ was used for the level of significance.

3. Results

3.1. Cytotoxicity

Viability of fibroblasts in dermal equivalents was assessed using the MTT assay. Exposure to *m*-xylene resulted in a time- and dose-dependent decrease in fibroblast viability after 1- or 4-h (Fig. 1). Using non-linear regression analysis of dermal equivalent viability plotted against the calculated fibroblast concentration, the EC_{50} were calculated to be 1481.1 ± 88.2 and 930.4 ± 32.5 μg *m*-xylene/g cells at 1 and 4 h, respectively (Table 1). The observed EC_{50} at 1 h was ≈60% higher than the EC_{50} at 4 h.

Table 1
EC_{50} values derived from viability curves of dermal equivalents exposed to *m*-xylene for 1 or 4 h in the presence or absence of *N*-acetylcysteine (NAC) or catalase (CAT)

Exposure type	Time (h)	EC_{50}[a]
m-Xylene	1	1481.1 ± 88.2
m-Xylene + 1 mM NAC	1	1807.1 ± 60.4*
m-Xylene + 5 mM NAC	1	1948.7 ± 164.1*
m-Xylene + 1000 U/ml CAT	1	2000.0 ± 185.2
m-Xylene	4	930.4 ± 32.5
m-Xylene + 1 mM NAC	4	1429.5 ± 66.2*
m-Xylene + 5 mM NAC	4	1180.6 ± 60.0*
m-Xylene + 1000 U/ml CAT	4	1013.4 ± 95.8

[a] Data are expressed as mean ± S.E.M. ($n = 4$).
* Data are significantly higher than corresponding control.

3.2. Endogenous catalase and thiol levels

Endogenous catalase activity in the living dermal equivalents exposed to *m*-xylene decreased in a temporal and dose-dependent manner (Fig. 2). At 1 and 4 h, catalase activity decreased significantly ($P < 0.05$) with increasing chemical concentration. The mean (±S.E.M.) observed changes in absorbance values at 240 nm per minute (normalized to protein), reflecting catalase activity in the

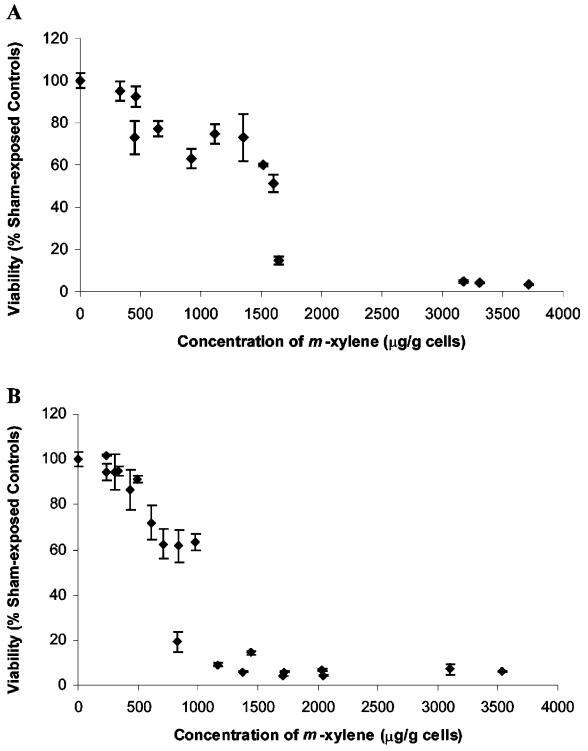

Fig. 1. Mean viability ± S.E.M. ($n = 4$ per sample) of dermal equivalents following a 1-h (A) or 4-h (B) exposure to *m*-xylene determined using the MTT assay. Values are expressed as a percentage of the sham-exposed controls.

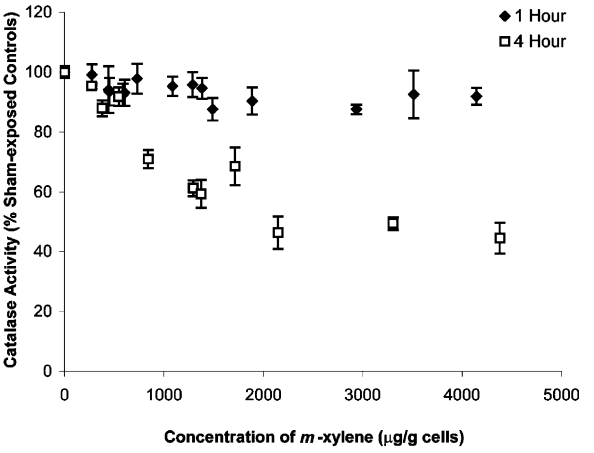

Fig. 2. Mean catalase activity ± S.E.M. ($n = 4$ per sample) in dermal equivalents following a 1- or 4-h exposure to *m*-xylene. Values are normalized to protein and expressed as a percentage of the sham-exposed controls.

sham-exposed controls, were 0.037 ± 0.001 and 0.042 ± 0.002 at 1 and 4 h, respectively. The maximum observed decreases in catalase activity were ≈ 10 and 55% at 1 and 4 h, respectively. The observed catalase activity was significantly lower ($P < 0.05$) at 4 h when compared to the 1 h time period.

DTNB-reactive thiol levels in the living dermal equivalents decreased in a dose-dependent manner following exposure to *m*-xylene (Fig. 3). The mean ($\pm$S.E.M.) observed absorbance values for DTNB-reactive thiols at 412 nm (normalized to protein) in the sham-exposed controls were 4.61 ± 0.37 and 4.29 ± 0.15 at 1 and 4 h, respectively. The maximum observed decreases in thiol levels were ≈ 20 and 40% at 1 and 4 h, respectively. At 1 h, thiol levels did not decrease significantly as a result of increasing chemical concentration. At 4 h, a significant decrease ($P < 0.05$) in thiol levels was observed with increasing chemical concentration. Thiol levels were significantly lower ($P < 0.05$) at 4 h when compared to the 1-h time period.

3.3. Effect of adding NAC and catalase

The concentrations of NAC and catalase used in this study were not cytotoxic to the dermal fibroblasts. Following 16–18 h incubation, the viability of cells exposed to NAC at concentrations

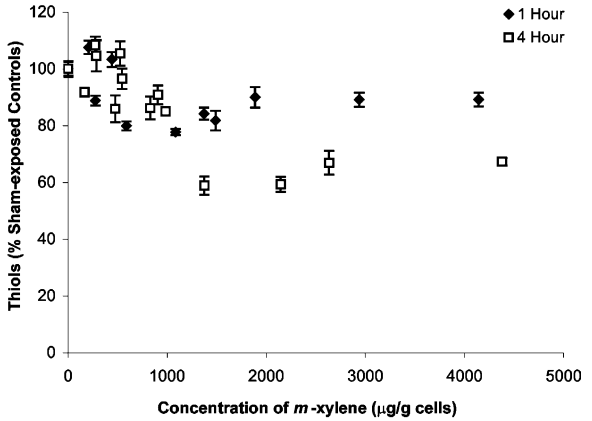

Fig. 3. Mean DTNB-reactive thiols $\pm$S.E.M. ($n = 4$ per sample) in dermal equivalents following a 1- or 4-h exposure to *m*-xylene. Values are normalized to protein and expressed as a percentage of the sham-exposed controls.

of 1.0 and 5.0 mM was 97.2 ± 4.3 and 103.6 ± 5.6% of sham-exposed controls. The viability of cells exposed to 1000 U/ml catalase was 98.3 ± 2.1% of sham-exposed controls.

When compared to *m*-xylene exposures, pre-treatment with either 1.0 or 5.0 mM NAC led to significant increases ($P < 0.05$) in the EC_{50} values at 1 and 4 h (Table 1). Increases in these EC_{50} values ranged from 1.22- to 1.32-fold at 1 h and 1.27- to 1.54-fold at 4 h. Although pre-treatment with catalase led to a 1.35-fold increase in cell viability at 1 h, no significant differences were observed at either 1 or 4 h when compared to dermal equivalents exposed to *m*-xylene alone.

4. Discussion

Exposure methods have been developed for in vitro assessment of volatile chemical-induced cytotoxicity (Rogers and McDougal, 2002). The headspace and culture medium volume, chemical solubility and chemical partitioning affect VOC dosimetry. A problem with conducting in vitro exposures using VOCs is maintaining these chemicals in solution to achieve a uniform exposure. Any selected in vitro exposure method should inhibit VOC evaporation from the culture medium, thereby preventing a temporal decrease in chemical concentration. We have previously observed such a temporal decrease in *m*-xylene from the culture medium in unsealed 24- and 48-well culture plates (Rogers and McDougal, 2002). This decrease in chemical concentration resulted in viability values that were 4- to 6-fold higher than viability values determined using our sealed system (Rogers and McDougal, 2002). The present study utilized a sealed exposure method previously developed in our laboratory to examine the effect of *m*-xylene exposure on dermal fibroblast viability and depletion of cellular antioxidants. Our results show that dermal fibroblasts exposed to *m*-xylene for 1 or 4 h exhibited a time- and dose-dependent decrease in cellular viability and endogenous thiols and catalase activity. Moreover, NAC pretreatment of dermal equivalents led to significant increases in cellular viability, while

catalase pretreatment promoted a marginal, but non-significant increase in cell viability.

Chemical exposure can deplete endogenous cellular thiol levels, leading to a decrease in cellular viability (Stanislawski et al., 2000; Cereser et al., 2001; Chang et al., 2001; Chiba et al., 2001; Chang et al., 2002). Endogenous thiols can protect cells from oxidative stress by direct free radical quenching. The predominant thiol-containing free radical scavenger is glutathione (GSH), which protects cells in vivo and in vitro against oxidative stress. The GSH redox cycle serves as the most important free radical-removing system in cells (Meister and Anderson, 1983). NAC is a stable thiol-containing compound that can protect cells against oxidative stress by itself or as a constituent used for GSH synthesis. Once NAC penetrates the cell membrane, it is readily deacetylated and provides cellular cysteine for GSH synthesis (Gillissen and Novak, 1998). The free –SH group of NAC itself can directly bind hydroxyl radicals or interact with H_2O_2 to form water and O_2 (Gillissen and Novak, 1998). NAC has been shown to provide protection against oxidative stress and cytotoxicity in cultured cells (Emonet et al., 1997; Jeng et al., 1999; Stanislawski et al., 2000; Cereser et al., 2001; Chang et al., 2002).

Although GSH is the predominant thiol-containing antioxidant in cells, there are additional endogenous thiol-containing compounds that can serve as potent free radical scavengers (Di Simplicio et al., 1998; Giustarini et al., 2000). Therefore, we chose to monitor decreases in the levels of cellular thiols (determined as DTNB-reactive) in dermal equivalents exposed to *m*-xylene. A decrease in DTNB-reactive thiols may reflect depletion of the free radical scavenging capacity of –SH groups. Such a decrease in thiols could lead to a build-up of free radicals inside the dermal fibroblasts. Our results show that exposure of dermal lattices to *m*-xylene leads to a temporal and dose-dependent decrease in endogenous thiols that occurred with decreasing cell viability. Similar results have been observed in cells exposed to UV light, thiram, arecoline, diethyl maleate and chlorodinitrobenzene (Aoshiba et al., 1999; Cereser et al., 2001; Chang et al., 2001, 2002). Pretreatment of the dermal equivalents with 1.0 or 5.0 mM NAC led to significant increases in cell viability at both 1 and 4 h. This protective effect of NAC against cytotoxicity has also been observed in fibroblasts exposed to dental resins, chemicals or UV light (Tyrrell and Pidoux, 1986; Emonet et al., 1997; Jeng et al., 1999; Stanislawski et al., 2000; Cereser et al., 2001). Our results suggest that endogenous thiols play a role in protecting dermal fibroblasts against *m*-xylene-induced cytotoxicity.

Catalase is an antioxidant enzyme that protects the cells against increases in hydrogen peroxide levels produced by the cell during normal and perturbed conditions (Makino et al., 1994, 1995; Masaki et al., 1998). Decreases in catalase activity have been identified in disease, aging and exposure to chemicals and UV light (Lu et al., 1999; Yan et al., 1999; Alam et al., 2000; Sander et al., 2002). In the present study, *m*-xylene exposure led to a time- and dose-dependent decrease in endogenous catalase in dermal fibroblasts that occurred with decreasing cell viability. The addition of catalase during *m*-xylene exposure appeared to provide marginal protection against cytotoxicity, since we observed that the presence of catalase promoted a non-significant increase in cell viability at 1 h with no effect on cell viability at 4 h. These results suggest that catalase may be providing little protection against *m*-xylene-induced cytotoxicity.

Oxidative stress produced following exposure to the free radical-generating stimuli, such as UV light, silica and 3-morpholinosydnonimine-*N*-ethylcarbamide, contributes to cytotoxicity in dermal fibroblasts (Masaki and Sakurai, 1997; Lomonosova et al., 1998; Cho et al., 1999). This cytotoxicity can be partially inhibited by the addition of catalase during exposure of the fibroblasts. Exogenous nitric oxide, hyperoxia conditions or sodium arsenite have also been observed to be cytotoxic to fibroblasts, which can be inhibited by supplementing catalase during exposure (Lee and Ho, 1995; Raghuram et al., 1999). However, such catalase supplementation does not always provide protection. Fibroblasts exposed to iron, nicotine or codeinone were not protected against cytotoxicity by catalase, but some protection was observed by using hydroxyl radical scavengers (such as NAC), suggesting that hy-

droxyl radicals may be primarily responsible for the observed cytotoxicity (Pu et al., 2001; Kawase et al., 2002). This could be an explanation for the results we observed in this study with respect to *m*-xylene-induced cytotoxicity in which NAC provided better cytotoxic protection than catalase. Since NAC itself can bind and interact with free radicals, this protection may be due to the direct action of NAC. Moreover, since NAC is a constituent used for GSH synthesis, protection against *m*-xylene-induced cytotoxicity could also be due to an increase in cellular GSH formed during the pretreatment of fibroblasts with NAC.

Skin irritation following exposure to chemicals or UV light appears to be modulated in part by oxidative stress (Yasui and Sakurai, 2000; Willis et al., 2001). Antioxidants applied to the skin can inhibit or reduce the degree of dermal irritation caused by chemical or UV light exposure (Senaldi et al., 1994; Bangha et al., 1996; Fuchs and Kern, 1998; Lange et al., 1998; Katiyar et al., 1999; Dreher and Maibach, 2001). The VOC *m*-xylene is considered an irritant and exposure to *m*-xylene can lead to skin irritation and inflammation, as well as the formation of oxidative species and DNA damage (Steele and Wilhelm, 1966, 1970; Rogers et al., 2001). The results of the present study show that *m*-xylene induces cytotoxicity and promotes decreases in cellular antioxidants (thiols and catalase) in fibroblasts in a time- and dose-dependent manner. The cytotoxicity observed at high chemical concentrations in which there was an observed marginal decrease ($\approx$10–20%) in endogenous levels of thiols and catalase may result from cellular damage other than oxidative stress. However, the observed significant decrease in cellular thiols and catalase from 1 to 4 h suggests that free radicals may be formed with increasing chemical concentration and exposure time, leading to depletion of thiols and catalase. Moreover, supplementation of the dermal equivalents with catalase or NAC appears to partially inhibit *m*-xylene-induced cytotoxicity, suggesting oxidative stress may play a role in the cytotoxic effect of *m*-xylene in rat dermal fibroblasts. Further studies on other antioxidant and/or cytotoxic mechanisms that play a role in the *m*-xylene-induced cytotoxicity need to be explored in order to understand the mechanism(s) underlying *m*-xylene-induced cytotoxicity in rat dermal fibroblasts. Once these mechanisms are known, it could be possible to develop therapeutic treatments for inhibiting or preventing the irritating effects of *m*-xylene and other VOCs.

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