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Differential response to acrylonitrile toxicity in rat primary astrocytes and microglia

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Abstract

Acrylonitrile (ACN) is a widely used chemical in the production of plastics, resins, nitriles, acrylic fibers, synthetic rubber and acrylamide. While acute high level exposures to ACN are known to be lethal, chronic low dose exposures causes glial cell tumors in rats. Recently, these glial tumors have been characterized as microglial in origin. While effects of ACN on astrocytes, the more numerous glial cell, have been investigated, the effects on microglia are unknown. This study was conducted to compare the responses of astrocytes and microglia to ACN treatment *in vitro* to address differential sensitivities and adaptive responses to this toxic chemical. Cell viability, ACN uptake, lipid peroxidation byproducts (F₂-isoprostanes), glutathione (GSH) levels and expression of NF-E2-related factor 2 (Nrf2) were evaluated in primary rat microglia and astrocytes following ACN treatment. Results indicate that microglia are more sensitive to ACN than astrocytes, accumulating less ACN while demonstrating higher F₂-isoprostane levels. GSH levels were up-regulated in both cell types, as a protective mechanism against ACN-induced oxidative stress, while Nrf2 levels were only induced in microglia. Our data suggest that microglia and astrocytes exhibit different sensitivities and responses to ACN, which are linked to the intracellular thiol status inherent to each of these cell types.

Keywords

Acrylonitrile; Astrocytes; Microglia; Glutathione; Oxidative stress

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Conflict of interest statement

None.

1. Introduction

Acrylonitrile (ACN) is an extensively used vinyl monomer used in the manufacturing of acrylic fibers, plastics, resins, nitriles, synthetic rubber and acrylamide. It is also an intermediate in the production of dyes and pharmaceuticals, and trace amounts have been found in cigarette smoke (Byrd et al., 1990; IHS, 2011). Human exposure to ACN is predominantly due to high level accidental exposures and low level chronic exposure to trace amounts of ACN. Acute exposures to high levels causing limb weakness, labored or irregular breathing, dizziness, impaired judgment, hallucinations, cyanosis, nausea, collapse, loss of consciousness, convulsions, and death (Buchter and Peter, 1984; Vogel and Kirkendall, 1984). Chronic exposure to ACN have been shown to cause liability of autonomic functions, such as lowered arterial pressure, labile pulse, diffuse dermatographia, increased sweating and change in orthostatic reflex have been observed in occupational exposures to ACN (Buchter and Peter, 1984). Similar effects have been observed in rats exposed to ACN, as well as the presence of primary brain tumors (Bigner et al., 1986; Kirman et al., 2005). Epidemiology data has not shown a direct correlation between ACN exposure and cancer in humans (Cole et al., 2008; IARC, 1999; Klaunig, 2008), prompting a classification as a group 2B possible carcinogen by the International Agency for Research on Cancer (IARC).

The majority of the brain tumors observed in rats following ACN exposure are gliomas (Bigner et al., 1986). Glial cells comprise around 90% of the total cells in the brain and comprise 50% of brain cellular volume (Allen and Barres, 2009; Parpura et al., 2012). Astrocytes are the predominant glial cells of the brain and are responsible for the regulation of ion concentrations in the extracellular fluid, development of tight junctions in the blood–brain barrier (BBB), differentiation of myelinated axons, neuro-transmitter uptake and release, metabolic support, nervous system repair, vasomodulation, and the promotion of myelination by oligodendrocytes (Allen and Barres, 2009; Parpura et al., 2012). The effects of ACN on astrocytes have been well established *in vitro*. Treatment of ACN in primary and transformed astrocytes leads to gliosis, increased reactive oxygen species (ROS), glutathione (GSH) and ATP depletion, increased oxidative DNA damage, and inhibition of gap junction intercellular communication (Enongene et al., 2000; Esmat et al., 2007; Jacob and Ahmed, 2003; Kamendulis et al., 1999a,b; Whysner et al., 1998). From these studies it is clear that oxidative stress plays a critical role in ACN-mediated neurotoxicity.

ACN toxicity has been linked to its metabolism. Conjugation of ACN to GSH is the predominant metabolic pathway (Fennell et al., 1991). An alternative pathway for ACN metabolism is *via* cytochrome P450 2E1 (CYP2E1), which converts ACN into an epoxide and releases cyanide (Guengerich et al., 1981; Wang et al., 2002). Cyanide toxicity and ACN toxicity share many of the same symptoms, such as limb weakness, irregular breathing, dizziness, nausea, collapse, and convulsions (Hamel, 2011; Thier et al., 2001). High exposures to ACN lead to increased oxidative stress by depleting cellular GSH, altering the cellular redox status, as well as shifting metabolism to the CYP2E1 pathway, resulting in increased cyanide production, which can lead to further production of ROS by inhibiting the mitochondrial respiratory chain. Stimulation of hepatic CYP2E1 activity with agonists has shown increased cyanide production and inhibition of cytochrome *c* oxidase (Complex IV)

in rat brains (Suhua et al., 2010). Increased ACN-induced oxidative stress has also been linked with cell death and dysfunction, as well as Bax mediated apoptosis in the SH-SH5Y cell line (Watcharasit et al., 2010). Treatment of rats or cells with the antioxidants curcumin, α -tocopherol, epigallocatechin gallate (EGCG), or N-acetyl- L-cysteine (NAC) efficiently attenuates ACN-induced oxidative damage, providing further evidence that ACN toxicity is primarily mediated through increased oxidative stress (Esmat et al., 2007; Guangwei et al., 2010; Zhang et al., 2000).

Recently, immunohistochemistry has shown that the ACN-induced gliomas in rats are comprised of microglia (Kolenda-Roberts et al., 2013). Microglia play important roles in inflammation and extracellular signaling; they also respond to neuronal injury by releasing both neurotoxic and neurotrophic factors (Aschner et al., 1999). All of these processes can be modulated by oxidative stress, thus compromising microglial function. We hypothesized that microglia may have different sensitivities and responses to ACN exposure than astrocytes, the more numerous glial cells. Our studies found that although there was no difference in cell viability following ACN treatment, microglia were more sensitive than astrocytes, taking up less ACN than astrocytes, but showing higher levels of oxidative stress and up-regulation of the oxidative stress responsive transcription factor, NF-E2-related factor 2 (Nrf2). These results highlight the importance of microglia in ACN toxicity.

2. Materials and methods

2.1. Reagents

Unless otherwise stated, all biochemical reagents used in this study were purchased from Sigma Chemicals (St. Louis, MO, USA).

2.2. Primary cell culture and treatments

Primary microglia and astrocytes were isolated from cortical tissue of postnatal day-1 neonatal Sprague-Dawley rats, as previously described (Ni and Aschner, 2010; Ni et al., 2011). After dissociation, centrifugation and resuspension, the mixed glial cell culture was maintained in 225 cm² tissue culture treated flasks fed with minimum essential medium (MEM; Invitrogen, Carlsbad, CA), supplemented with 10% heat-inactivated horse serum (Invitrogen), 100 U/ml penicillin and 100 μ g/ml streptomycin. The cultures were maintained in a humidified incubator at 37 °C of 5% CO₂. The media were changed twice a week. After 2 weeks in culture, when cells reached ~95% confluence, microglia and astrocytes were separated by gentle shaking for 20 min at room temperature. Using this protocol, our lab has achieved 95% purity of the isolated microglia and astrocytes, as verified by immunostaining for glial fibrillary acidic protein (GFAP) for astrocytes and OX42 for microglia (Ni and Aschner, 2010; Ni et al., 2011). Equal density of microglia and astrocytes were plated in poly-L-lysine coated plates (BD Biocoat, San Jose, CA). Three days after the cell attachment, cells were exposed to 0, 0.1, 0.5, 0.75 or 1 mM ACN (Sigma–Aldrich) for different durations.

2.3. Cytotoxicity assays

The *in vitro* cytotoxic effects of ACN were evaluated with the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) and lactate dehydrogenase (LDH) toxicity assay kits (TOX1 and TOX7, Sigma –Aldrich) as per the manufacturer's instructions. The MTT assay was used as a measure of viable cells, as the reduction of MTT takes place in active living cells, whereas the LDH assay was used as a measure of dead cells; LDH is leaked upon death and membrane compromise. The absorbance for MTT assay was measured at 570 nm and LDH assay at 490 nm. The background absorbance (690 nm) was subtracted out for both assays.

2.4. Radiolabeled ACN uptake assay

Equal density of microglia and astrocytes were plated in poly-L-lysine coated 24-well plates. Three days after the cell attachment, cells were exposed to 0, 0.1, 0.5, 0.75 or 1 mM ACN with 0.25 μ Ci/ml of 14 C (American Radiolabeled Chemicals, ARC 1745-1 mCi/37 MBq, St. Louis, MO) for 1, 5, 10 and 30 min. The uptake assay was then performed following a published protocol (Sidoryk-Wegrzynowicz et al., 2011). Briefly, the cells were washed two times with cold freshly prepared sodium-HEPES buffer with a pH of 7.4 to stop the treatment. Cells were then lysed with 1 M NaOH for 30 min at room temperature. A portion of cell lysate was transferred to scintillation vials and mixed with 4 ml ScintiSafe 30% Cocktail (Fisher Scientific, Pittsburgh, PA) and radioactivity was measured by a scintillation counter (LS 6500 Beckman). Protein concentration was determined using the bicinchoninic acid (BCA) assay (Pierce, Rockford, IL) with bovine serum albumin as the standard.

2.5. F₂-isoprostanes quantification

After treatment with ACN, cells were rapidly harvested, flash frozen in liquid nitrogen and stored at -80°C until use. Total F₂-isoprostanes were measured following a published protocol using a stable isotope dilution method with detection by gas chromatography/mass spectrometry (GC/MS) and selective ion monitoring (Milatovic et al., 2005; Morrow and Roberts, 1994). Bradford protein assay with bovine serum albumin as the standard was performed for the normalization of isoprostane quantification.

2.6. Measurement of reduced glutathione and oxidized glutathione

After ACN treatment, cells were washed once with $1 \times$ Phosphate Buffered Saline solution and then harvested with 5% perchloric acid/0.2 M boric acid solution on ice. The reduced GSH and GSSG concentrations were measured by high-performance liquid chromatography (HPLC) as previously described (Ni et al., 2011; Wang et al., 2009). GSH and GSSG concentrations were normalized to the protein concentration of the samples, determined by the BCA assay.

2.7. Western blot analysis

Following ACN treatment, cells were scraped and collected in radioimmunoprecipitation assay (RIPA) buffer with protease inhibitor cocktail on ice. Western blot was performed with the primary antibody of rabbit-anti Nrf2 (1:200) (sc-722, Santa Cruz Biotechnology, Santa Cruz, CA) and secondary antibody of goat anti-rabbit IgG (1:5000) (31460, Thermo

Scientific, Rockford, IL) to detect Nrf2 protein. β -Actin was detected by applying primary antibody of mouse anti β -actin (1:100,000) (A5316, Sigma) and second antibody of goat anti-mouse IgG (1:5000) (474-1806, KPL, Gaithersburg, MD). The density of the Nrf2-specific bands was normalized to β -actin.

2.8. Statistical analysis

All results were expressed as means $\pm$ standard errors with a minimum of three independent experiments. One-way analysis of variance (ANOVA) and two-way ANOVA followed by Bonferroni's *post hoc* test were performed using Prism 4 (Graphpad Software Inc., San Diego, CA). Values of $P < 0.05$ were considered statistically significant.

3. Results

3.1. ACN is not cytotoxic to astrocytes or microglia

To compare the sensitivity of rat primary microglia and astrocytes to ACN, cell viability was examined with MTT or LDH assays. Equal densities of cells (20,000 cells/well) were treated with increasing concentrations of ACN (0.1, 0.5, 0.75, and 1 mM) for 24 h. Both microglia and astrocytes showed no significant loss of viability in response to ACN treatment by either the LDH (Fig. 1A) or MTT (Fig. 1B) assays. This suggests concentrations of 1 mM or less do not affect cell viability in astrocytes or microglia.

3.2. Astrocytes accumulate more ACN than microglia

To determine whether there was differential uptake of ACN in microglia and astrocytes, both cell types were treated with ^{14}C radio-labeled ACN (0.1, 0.5, 0.75, and 1 mM) for 1, 5, 10, and 30 min, washed, and lysed to determine the amount of radioactive ACN contained in the cells. In Fig. 2, both astrocytes and microglia showed a time-dependent accumulation of ACN. ACN content significantly increased in microglia starting at 10 min and continued for 30 min after 0.75 or 1 mM ACN treatment as compared to the 1 min untreated controls. However, ACN uptake was only significant in microglia after 30 min of treatment with 0.5 mM ACN as compared to the 1 min control. Uptake of ACN (0.5, 0.75, and 1 mM) by astrocytes was significant after 10 and 30 min of exposure as compared to the 1 min astrocyte control. Astrocytes also accumulated significantly more ACN than microglia after 30 min of exposure following treatment with 0.5, 0.75, and 1 mM ACN. This suggests that astrocytes may act as a sink, taking up larger amounts of ACN than the microglia.

3.3. Differential levels of lipid peroxidation in microglia and astrocytes following ACN exposure

ACN has been shown to cause oxidative stress in rat brain, neuronal cell lines, and primary glial cultures, with increased oxidative DNA damage and GSH depletion (Kamendulis et al., 1999a; Pu et al., 2006; Watcharasi et al., 2010). F_2 -isoprostanes are generated from the process of free-radical peroxidation of arachidonic acid (AA) and are long lived markers of oxidative stress (Milne et al., 2005). As F_2 -isoprostanes have not been measured in response to ACN, we were interested in determining whether these compounds formed in either primary rat astrocytes or microglia. F_2 -isoprostanes were measured in whole cell extracts from microglia or astrocytes treated with 0.1, 0.5, 0.75, or 1 mM ACN for 24 h. ACN

treatment (0.75 and 1 mM) resulted in significant increases in F₂-isoprostanes in microglia above non-treated controls (Fig. 3). Conversely, astrocytes treated with ACN showed no changes in F₂-isoprostane levels after 24 h of exposure as compared to non-treated control cells. This data suggests that microglia may be more sensitive than astrocytes to the oxidative stress induced by ACN.

3.4. Increased GSH and Nrf2 levels after ACN exposure

A potential explanation for astrocytes producing less F₂-isoprostanes than microglia is that the two cell types have different basal GSH levels (Ni et al., 2011), therefore their GSH redox ratios respond differently to ACN exposure. ACN has been shown to undergo Michael addition reactions with nucleophiles, such as GSH (Woutersen, 1998). High exposures of ACN can lead to depletion of GSH and oxidative stress (Woutersen, 1998). Previous observations have shown that GSH content of the cortex, cerebellum, and hippocampus are decreased in rats exposed to ACN (Rongzhu et al., 2009), however it is unclear how ACN affects GSH content of specific cell types, such as microglia. Microglia and astrocytes were treated with ACN (0.1, 0.5, 0.75, and 1 mM) for 24 h and total GSH was immediately extracted and measured by HPLC. In Fig. 4A, reduced GSH levels were increased in both astrocytes and microglia following 0.5, 0.75, and 1 mM ACN treatments, with significantly higher GSH levels in astrocytes than in microglia. After 24 h of ACN treatment, oxidized GSH, GSSG, levels were only increased in astrocytes treated with 1 mM ACN (Fig. 4B). This suggests that 24 h after exposure to ACN, both microglia and astrocytes up-regulate intracellular GSH levels to compensate for any GSH depletion that occurs immediately after exposure.

As GSH levels are regulated by both its depletion and synthesis, we next investigated induction of Nrf2, the transcription factor responsible for the expression of both subunits of the rate limiting enzyme in GSH synthesis, γ -glutamate cysteine ligase (GCLc and GCLm) (Baird and Dinkova-Kostova, 2011). Whole cell extracts from microglia showed a concentration-dependent up-regulation of Nrf2 levels after 24 h of ACN exposure, with increased expression of Nrf2 above untreated control following 0.75 and 1 mM exposure to ACN (Fig. 5). In contrast, Nrf2 levels were unchanged in astrocytes following 24 h exposure to ACN (Fig. 5). Our lab has previously observed differential induction of Nrf2 kinetics in astrocytes and microglia in response to methylmercury (MeHg) (Ni et al., 2011). As with MeHg exposure, microglia are more sensitive to ACN treatment than astrocytes, showing significant up-regulation of Nrf2 after 24 h of treatment while astrocytes display none.

4. Discussion

ACN is a widely used nitrile that has been shown to cross the blood–brain barrier and cause toxicity in the CNS of rats (Ahmed et al., 1996). Astrocytes have been extensively studied as targets of ACN toxicity, primarily due to their abundance and the presence of gliomas in rodents exposed to ACN (Bigner et al., 1986). Recent research has identified microglia as being the predominant cells in ACN-induced gliomas (Kolenda-Roberts et al., 2013), highlighting the importance of this cell type. Microglia are the resident macrophages of the brain responsible for responding to injury and inflammation. Astrocytes have been shown to

secrete inflammatory mediators, such as tumor necrosis factor- α (TNF- α), following ACN exposure, and neutrophil infiltration has been shown to be involved in ACN-induced gastric mucosal damage (Hamdy et al., 2012; Jacob and Ahmed, 2003), suggesting a role for inflammatory cells in ACN-induced toxicity. To our knowledge, this is the first study to investigate the effects of ACN on primary microglia and compare their responses to primary astrocytes treated with ACN. Based on several measurements, we observed that microglia were more sensitive to ACN than astrocytes. This is consistent with previous studies showing microglia were more sensitive than astrocytes to MeHg and trimethyltin (Monnet-Tschudi et al., 1995; Ni et al., 2011).

In this study, ACN concentrations ranging from 0.1 to 1 mM ACN produced no cell death in either astrocytes or microglia after 24 h as measured by two methods of assessing cell viability (Fig. 1). ACN is cytotoxic in neuronal cell lines, causing an activation of Bax and induction of apoptosis (Watcharasit et al., 2010). Jacob and Ahmed found significant cell death in primary normal human astrocytes treated with 200 and 400 μ M ACN, while Pu et al. have observed 1 mM ACN to be a sublethal exposure in primary human astrocytes (Jacob and Ahmed, 2003; Pu et al., in preparation). Comparisons between primary rat and human astrocytes viability after ACN exposure is complex due to species differential responses to ACN. ACN has been shown to increase GFAP, a marker of astrogliosis, in rats, and also to increase number of transformed colonies in Syrian hamster embryo model of cell transformation by chemical carcinogens (Enongene et al., 2000; Zhang et al., 2000). While our primary astrocyte cultures showed no significant cell death, proliferation was not investigated (Fig. 1). The effects of ACN on microglia *in vitro* have not been previously documented, and needs to be further characterized.

Oxidative stress plays a major role in ACN-mediated toxicity. Several studies have noted an increase in ROS production in neuronal and astrocytic cell lines following ACN treatment (Jacob and Ahmed, 2003; Kamendulis et al., 1999a; Watcharasit et al., 2010). ACN has also been shown to inhibit the activities of antioxidant enzymes, such as superoxide dismutase (SOD) and catalase, contributing to the increased oxidizing environment (Guangwei et al., 2010; Kamendulis et al., 1999a; Zhang et al., 2002). Many of these effects can be blocked by antioxidants (Esmat et al., 2007; Guangwei et al., 2010; Zhang et al., 2000), emphasizing the role of oxidative stress in ACN-induced toxicity. We observed that primary rat astrocytes and microglia accumulate ACN (Fig. 2), but that astrocytes take up significantly more ACN than microglia on a per cell basis. Based on this observation it was expected that there would be increased oxidative stress in both cell lines, with higher levels detected in astrocytes. Surprisingly, F₂-isoprostanes, markers of lipid peroxidation and oxidative stress, were only significantly increased in microglia (Fig. 3), suggesting that these cells are more sensitive to ACN. We have previously observed significantly less reduced GSH, and thus less reducing potential, in primary rat microglia as compared to astrocytes (Ni et al., 2011). Having a lower thiol pool might prime microglia to be more sensitive to the ACN-induced oxidative stress. Oxidative stress-induced DNA lesions have been observed in astrocytic cell lines and white blood cells of rats exposed to ACN (Kamendulis et al., 1999a; Pu et al., 2006, 2009), however levels of these lesions have not been measured in microglia. Based on our observation of increased oxidative stress in microglia compared to astrocytes, microglia may have a higher level of oxidative DNA damage, which could lead to microgliosis.

The distinct sensitivities of microglia and astrocytes indicate different roles for the two cell types in responding to ACN exposure. Both astrocytes and microglia showed an increase in reduced GSH content following ACN treatment (Fig. 4), but there was significantly less up-regulation in microglia than in astrocytes. The amount of up-regulation of reduced GSH in astrocytes is quite remarkable; we observed around 6-fold increase after exposure to ACN as compared to 3-fold induction of GSH following exposure of astrocytes to the neurotoxicant 6-hydroxydopamine or the antioxidants tert-butylhydroquinone and curcumin (Eftekharpour et al., 2000; Lavoie et al., 2009; Zhang et al., 2005). The increase in GSH in astrocytes may be due to their involvement in metabolizing ACN *via* GSH conjugation and the CYP2E1 metabolic pathway. We have observed that ACN accumulates more in astrocytes and it is known that these cells express CYP2E1 (Montoliu et al., 1995), however it is unknown whether microglia express CYP2E1. Additionally astrocytes and microglia express multidrug resistance-associated protein 1 (Mrp 1, ABCC1), an ATP-driven pump that mediates export of organic anions and GSH conjugates (Gibson et al., 2012; Mercier et al., 2004). Astrocytes are closely associated to the BBB, allowing for ACN–GSH conjugates to be removed from the brain by Mrp 1 or other related transporters. Since GSH conjugation is the preferred pathway (Fennell et al., 1991), high exposures to ACN lead to depletion of cellular GSH, which alters the cellular redox status. As we observed increased lipid peroxidation markers in microglia (Fig. 3), it was expected that we may also observe GSH depletion following ACN treatment, which has also been observed in rat primary glial cells exposed to 1 mM ACN as early as 3 h (Esmat et al., 2007). Additionally, Kamendulis et al. has found GSH levels to be significantly decreased following similar treatment conditions of 1 mM ACN for 24 h in the DITNC1 rat astrocyte cell line (Kamendulis et al., 1999a). Our data show that 24 h after exposure levels of reduced GSH were increased above basal levels in both primary astrocytes and microglia, indicating a protective mechanism to prevent oxidative stress (Fig. 4). Levels of reduced GSH following ACN treatment were elevated to the extent that there was only an increase in oxidized GSH in astrocytes at the highest ACN concentration. The differences between Kamendulis et al. and our findings may be due to use of cell line *vs.* primary cultures.

Surprisingly, despite both astrocytes and microglia showing an increased level of GSH in response to ACN, levels of Nrf2 were drastically different. Nrf2 is activated by oxidative stress to induce the transcription of antioxidant response genes, including heme oxygenase 1 (HO-1), NAD(P)H dehydrogenase, quinone 1 (NQO1), and γ -glutamate cysteine ligase (subunits GCLc and GCLm) (Baird and Dinkova-Kostova, 2011). GCL is the rate limiting step in GSH synthesis, and would be expected to be up-regulated when GSH levels are increased. In microglia Nrf2 levels were increased following ACN treatment, suggesting that the ACN-induced oxidative stress activated the transcription factor. Astrocytes did not show an increase in Nrf2 levels in response to ACN despite the increased GSH content (Fig. 5). This is not surprising considering that lipid peroxidation markers were not observed in these cells following ACN treatment. Dynamics of Nrf2 induction has been shown to be different in astrocytes and microglia, with astrocytes showing slower induction in response to several types of environmental contaminants, including MeHg, TiO₂, and trimethyltin (Kuhlmann and Guilarte, 2000; Long et al., 2007; Ni et al., 2011). While early time points were not investigated for ACN treatment in this study, the presence of increased Nrf2 in microglia

after 24 h (Fig. 5) suggests a prolonged response to the oxidative stress, which is not seen in astrocytes. It is important to note that Nrf2 activity is not entirely dependent on its levels, but rather its cellular localization. Nrf2 is largely compartmentalized to the cytoplasm by the Keap1 protein. Upon activation by oxidative stress and other signals, Nrf2 dissociates from Keap1 and translocates to the nucleus where it binds to antioxidant response elements (ARE) on DNA (Baird and Dinkova-Kostova, 2011). The possibility that Nrf2 is activated in astrocytes in response to ACN cannot be ruled out, as Nrf2 localization was not investigated in this study. Especially in microglia, activation of Nrf2 may be a protective mechanism to combat the ACN-induced oxidative stress.

In conclusion, our findings demonstrate differential response of microglia and astrocytes to ACN. Astrocytes accumulated ACN to a greater extent than microglia and maintained higher levels of intracellular GSH, possibly to compensate for GSH depletion caused by the increased uptake of ACN. Conversely, microglia accumulated less ACN, showed increased lipid peroxidation markers, increased GSH content and activation of Nrf2. The increase in F₂-isoprostanes and prolonged induction of Nrf2 suggest that microglia may be more sensitive to the ACN-induced oxidative stress, and are more primed by their lower thiol status to respond to ACN exposure, well before the astrocytes. The role microglia play in mediating ACN-induced neurotoxicity and whether ACN causes microglial activation remains to be determined. Previous *in vivo* data has focused on neurons and astrocytes, while markers for microglia have been overlooked. Future *in vivo* studies will help delineate the functions of astrocytes and microglia in ACN toxicity.

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Abbreviations

AA	arachidonic acid
ACN	acrylonitrile
ARE	antioxidant response element
BBB	blood–brain barrier
BCA	bicinchoninic acid
BSO	buthionine sulfoximine
CNS	central nervous system
CYP2E1	cytochrome P450 2E1
EGCG	epigallocatechin gallate
HPLC	high-performance liquid chromatography

GCL	γ -glutamate cysteine ligase
GC/MS	gas chromatography/mass spectrometry
GFAP	glial fibrillary acidic protein
GSH	glutathione
HO-1	heme oxygenase 1
IARC	International Agency for Research on Cancer
LDH	lactate dehydrogenase
MeHg	methylmercury
MEM	minimum essential media
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide
NAC	N-acetyl-L-cysteine
NQO1	NAD(P)H dehydrogenase, quinone 1
Nrf2	NF-E2-related factor 2
RIPA	radioimmunoprecipitation assay
ROS	reactive oxygen species
SOD	superoxide dismutase
TNF-α	tumor necrosis factor- α

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- Primary rat astrocytes and microglia were compared for their response to acrylonitrile.
- Microglia accumulated less acrylonitrile and showed increased markers of oxidative stress compared to astrocytes.
- Microglia appear to be more sensitive to acrylonitrile.

Fig. 1.

ACN treatment is not cytotoxic to rat primary microglia or astrocytes. (A) Cytotoxicity to ACN was measured in rat primary microglia and astrocytes with the LDH assay after ACN treatment for 24 h. Values are expressed as the mean $\pm$ SEM from 9 independent experiments. (B) Cytotoxicity to ACN was also measured in microglia and astrocytes with the MTT assay after ACN treatment for 24 h. Values are expressed as the mean $\pm$ SEM from 9 independent experiments.

Fig. 2.

Uptake of ACN is greater in astrocytes than microglia. Total ^{14}C -labeled ACN uptake was measured in microglia and astrocytes treated with increasing concentrations of ACN for 1, 5, 10, and 30 min. Data represent the mean $\pm$ SEM from 6 separate experiments. * $P < 0.05$, ** $P < 0.01$, and *** $P < 0.001$ vs. untreated microglia or astrocyte control. +++ $P < 0.001$ for astrocyte vs. microglia treatment group.

Fig. 3. F₂-isoprostanes are increased in microglia but not astrocytes after ACN treatment. Both cell types were treated with increasing concentrations of ACN for 24 h and F₂-isoprostanes were measured in whole cell extracts. Values represent mean $\pm$ SEM from 9 independent experiments. *** $P < 0.001$ vs. microglia control.

Fig. 4.

ACN treatment increases intracellular glutathione in both microglia and astrocytes. (A) Reduced GSH and (B) GSSG concentrations were measured by HPLC from cells treated with increasing concentrations ACN for 24 h. Values represent the mean $\pm$ SEM from 11 independent experiments expressed as % of the microglia untreated control. * $P < 0.05$, ** $P < 0.01$, and *** $P < 0.001$ vs. untreated microglia or astrocyte control. + $P < 0.05$, ++ $P < 0.01$, and +++ $P < 0.001$ for astrocyte vs. microglia treatment group.

Fig. 5.

Nrf2 protein levels are increased by ACN exposure in microglia but not astrocytes. Both cell types were treated with increasing concentrations of ACN for 24 h. The Nrf2 protein levels in whole cell lysates was measured by western blot analysis (representative of 5 separate experiments). β -Actin was used as a loading control. * $P < 0.05$ vs. microglia control.