NeuroToxicology 42 (2014) 1–7

Contents lists available at ScienceDirect

NeuroToxicology

Differential inflammatory response to acrylonitrile in rat primary astrocytes and microglia

Samuel W. Caito [a], Yingchun Yu [b], Michael Aschner [a,*]

[a] Department of Molecular Pharmacology, Albert Einstein College of Medicine, Bronx, NY, United States
[b] Division of Clinical Pharmacology and Pediatric Toxicology, Department of Pediatrics, Vanderbilt University Medical Center, Nashville, TN, United States

ARTICLE INFO

Article history:
Received 17 December 2013
Accepted 18 February 2014
Available online 11 March 2014

Keywords:
Acrylonitrile
Astrocytes
Microglia
Inflammation
NF-κB
p53

ABSTRACT

Acrylonitrile (ACN) is extensively used in the production of plastics, resins, nitriles and other commercial products. Chronic low dose exposures to ACN cause glial cell tumors in rats, primarily microglial in origin. Recently it has been determined that astrocytes and microglia respond to ACN-induced oxidative stress differently, which may influence cell-specific activation of inflammatory and carcinogenic pathways. This study was conducted to compare the inflammatory responses of astrocytes and microglia following ACN treatment *in vitro* to further characterize differential sensitivities and adaptive responses in these cell types. Nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) and p53 levels were measured along with levels of 12 different cytokines and chemokines in primary rat microglia and astrocytes. Additionally levels of cytochrome P450 2E1 (CYP2E1) were measured to evaluate the cells' ability to metabolize ACN. Results indicate that while both cells upregulate p53 and NF-κB, the cytokines and chemokines produced differ between the cell types. Astrocytes, but not microglia, upregulated CYP2E1 in response to ACN, which may be due to the astrocytes accumulating more ACN than the microglia. Altogether our data implicate the inflammatory response as an important event in ACN-induced neurotoxicity.

1. Introduction

Acrylonitrile (ACN) is a high volume vinyl monomer used as an intermediate during the synthesis of multiple products, including dyes, plastics, resins, nitriles, acrylic fibers, synthetic rubber and acrylamide, and is also found in cigarette smoke (Byrd et al., 1990; IHS, 2011) and when biomass is burned. Human exposure to ACN predominantly occurs in the occupational setting. Acute exposures to high levels of ACN cause 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 has been shown to cause lability of autonomic functions, such as labile pulse, lowered arterial pressure, diffuse dermographia, increased sweating and change in orthostatic reflex (Buchter and Peter, 1984). Studies in rats have shown similar effects, as well as the presence of primary brain tumors (Bigner et al., 1986; Kirman et al., 2005). No excess in cancers of any type has been observed in workers occupationally exposed to ACN (Cole et al., 2008; IARC, 1999; Klaunig, 2008), prompting a classification as a group 2B "possibly carcinogenic to humans" by the International Agency for Research on Cancer (IARC, 1999).

The majority of the brain tumors observed in rats following ACN exposure are gliomas (Bigner et al., 1986), which immunohistochemistry has shown to be comprised primarily of microglia (Kolenda-Roberts et al., 2012). Microglia respond to neuronal injury and play important roles in inflammation and extracellular signaling (Aschner et al., 1999). These cells can also be activated and attracted by astrocytes, the predominant glial cells of the brain that are responsible for a variety of functions, including metabolic support, nervous system repair and vasomodulation (Allen and Barres, 2009; Parpura et al., 2012). Many of the cellular processes

Abbreviations: ACN, acrylonitrile; CYP2E1, cytochrome P450 2E1; GFAP, glial fibrillary acidic protein; GM-CSF, granulocyte–macrophage colony-stimulating factor; GSH, glutathione; IFNγ, interferon γ; IL, interleukin; NF-κB, nuclear factor kappa-light-chain-enhancer of activated B cells; RANTES, chemokine (C–C motif) ligand 5/regulated on activation, normal T cell expressed and secreted; ROS, reactive oxygen species; TNF-α, tumor necrosis factor-α.

* Corresponding author at: Albert Einstein College of Medicine, 1300 Morris Park Avenue, Forchheimer Building, Room 209, Bronx, NY 10461, United States.
Tel.: +1 718 430 2317; fax: +1 718 430 8922.
E-mail address: michael.aschner@einstein.yu.edu (M. Aschner).

http://dx.doi.org/10.1016/j.neuro.2014.02.006
0161-813X/

regulated by astrocytes and microglia are important in the development of cancer, including inflammation, production of neurotrophic factors and angiogenesis. Chemical-induced carcinogenesis is a many-stepped process, relying on transformation of healthy cells into a cancerous phenotype. Exploring how ACN affects microglia and astrocytes inflammatory pathways is important to determine how these cells contribute to ACN-induced neurotoxicity and carcinogenesis.

The acute effects of ACN on astrocytes and microglia have been characterized *in vitro*. Previously, our laboratory has highlighted differences in response to ACN between the two cell types (Caito et al., 2013). Primary rat astrocytes were shown to accumulate ACN to a greater extent than primary rat microglia and maintained higher levels of intracellular glutathione (GSH). Conversely, microglia accumulated less ACN, showed increased lipid peroxidation markers, increased GSH content and activation of NF-E2-related factor 2 (Nrf2), suggesting that microglia may be more sensitive to ACN (Caito et al., 2013). Additionally, treatment of ACN in primary and transformed astrocytes leads to increased reactive oxygen species (ROS), ATP depletion and increased oxidative DNA damage (Enongene et al., 2000; Esmat et al., 2007; Jacob and Ahmed, 2003; Kamendulis et al., 1999a,b; Whysner et al., 1998), all of which are important inducers of both inflammatory and carcinogenic pathways.

Little is known about the ability of ACN to promote inflammation in the brain. Neutrophils have been found to accumulate and respond to inflammatory cues in ACN-induced mucosal damage in rats (Hamdy et al., 2012), however it is unknown whether the gliosis observed in rat brains following ACN exposure is due to activation of inflammatory cascades. Inflammation is a driving force for tumorigenesis; recruitment and activation of inflammatory cells results in angiogenesis and the release of proinflammatory mediators, many of which are hijacked by cancer cells for survival pathways (Coussens and Werb, 2002; Reuter et al., 2010). As both microglia and astrocytes can release proinflammatory mediators, and thus initiate an inflammatory response, we examined the hypothesis that due to their individual sensitivities to ACN, microglia and astrocytes may differ in activation of inflammation, as measured by the proinflammatory transcription factor NF-κB levels and cytokine release profiles. Furthermore, we investigated whether astrocytes and microglia utilized the same metabolic cytochrome P450 2E1 (CY2E1) pathway known to detoxify ACN and to produce cyanide and ROS, which could further trigger inflammation. Finally, we investigated whether astrocytes and microglia activate the p53 pathway, which is known to respond to oxidative DNA damage.

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 (Caito et al., 2013; Ni and Aschner, 2010; Ni et al., 2011). After dissociation, centrifugation and resuspension, the mixed glial cell culture was maintained in 225 cm^2 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 μg/ml streptomycin. The cultures were maintained in a humidified incubator at 37 °C with 5% CO_2. 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 was plated in poly-L-lysine coated plates (BD Biocoat, San Jose, CA). Three days after attachment, the cells were exposed to 0, 0.1, 0.5, or 1 mM ACN for 3 or 7 days.

2.3. Cytokine analysis

After exposure to ACN, cell culture media was collected for levels of proinflammatory mediators (interleukin (IL)-1α, IL-1β, IL-2, IL-4, IL-6, IL-10, IL-12, IL-13, interferon γ (IFNγ), tumor necrosis factor-α (TNF-α), granulocyte–macrophage colony-stimulating factor (GM-CSF), and chemokine (C–C motif) ligand 5/regulated on activation, normal T cell expressed and secreted (RANTES)) were measured using a rat inflammatory cytokine multi-analyte ELISArray kit (Qiagen, Hilden, Germany, catalog no. MER-004A), following the manufacturer's instructions using a SpectraMax M5 microplate reader (Molecular Device, Sunnyvale, CA).

2.4. 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 following primary antibodies: cytochrome P450 2E1 (Abcam, Cambridge, MA, catalog no. ab28146; 1:1000), phospho-p53 (ser15), p53 and RelAp65 (Cell Signaling Technology, Danvers, MA; catalog no. 9284, 2524, and 8242S respectively; 1:1000) and species appropriate secondary antibodies (goat anti-mouse IgG (1:5000) (474-1806, KPL, Gaithersburg, MD) and goat anti-rabbit IgG (1:5000) (31460, Thermo Scientific, Rockford, IL)). β-Actin was selected for a loading control and detected with a mouse anti β-actin antibody (1:100,000) (A5316, Sigma). The densities of the phospho-p53, p53 and p65-specific bands were normalized to β-actin.

2.5. Statistical analysis

All results were expressed as means ± 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. Induction of CYP2E1 by ACN in astrocytes

To determine whether the CYP2E1 metabolic pathway is involved in the response to ACN in microglia and astrocytes, we measured CYP2E1 levels in whole cell extracts from cells treated with ACN (0, 0.1, 0.5 or 1 mM) for either 3 or 7 days to simulate a repeated exposure. Both astrocytes and microglia express CYP2E1 under basal conditions (Fig. 1A and B). Astrocytes significantly and concentration-dependently increase CYP2E1 protein expression following treatment with ACN for 3 or 7 days (Fig. 1A and B). There is a trend for microglia to increase CYP2E1 protein expression following 3 days of ACN treatment, however it is not statistically significant (Fig. 1A). Following 7 days of treatment there is a significant increase of CYP2E1 in microglia treated with 1 mM ACN