G Model
TOXLET-8637; No. of Pages 10
ARTICLE IN PRESS
Toxicology Letters xxx (2014) xxx–xxx
Contents lists available at ScienceDirect
Toxicology Letters
journal homepage: www.elsevier.com/locate/toxlet

Immediate and delayed effects of subchronic Paraquat exposure during an early differentiation stage in 3D-rat brain cell cultures

Jenny Sandström von Tobel[a,b,*], Debora Zoia[b], Jorane Althaus[b], Paola Antinori[a,c], Julien Mermoud[b], Hui Song Pak[c], Alexander Scherl[a,c], Florianne Monnet-Tschudi[a,b]

[a] Swiss Centre for Applied Human Toxicology (SCAHT), Switzerland
[b] Department of Physiology, University of Lausanne, CH-1005 Lausanne, Switzerland
[c] Department of Human Protein Sciences, University of Geneva, CH-1200 Geneva, Switzerland

HIGHLIGHTS

- Immediate and longterm effects of paraquat were examined in 3D brain cell cultures.
- Paraquat was applied subchronically during an early neurodifferentiation stage.
- Neurons partially recovered from the paraquat insult after a 20-day washout period.
- Paraquat-induced astrogliosis persisted and inflammatory response changed over time.
- Paraquat caused delayed microglial activation of neurodegenerative M1-phenotype.

ARTICLE INFO

Article history:
Received 4 November 2013
Received in revised form 30 January 2014
Accepted 2 February 2014
Available online xxx

Keywords:
Paraquat dichloride
Microglial activation
M1/M2 phenotype
Astrogliosis
Neuroinflammation

ABSTRACT

Xenobiotic exposure is a risk factor in the etiology of neurodegenerative disease. It was recently hypothesized that restricted exposure during brain development could predispose for a neurodegenerative disease later in life. As neuroinflammation contributes to progressive neurodegeneration, it is suspected that neurodevelopmental xenobiotic exposure could elicit a neuroinflammatory process, which over time may assume a detrimental character.

We investigated the neurotoxic effects of paraquat (PQ) in three-dimensional whole rat brain cell cultures, exposed during an early differentiation stage, comparing immediate effects—directly post exposure—with long-term effects, 20 days after interrupted PQ-administration. Adverse effects and neuroinflammatory responses were assessed by measuring changes in gene- and protein-expression as well as by determining cell morphology changes.

Differentiating neural cultures were highly susceptible to PQ and showed neuronal damage and strong astrogliosis. After the 20-day washout period, neurons partially recovered, whereas astrogliosis persisted, and was accompanied by microglial activation of a neurodegenerative phenotype.

Our data shows that immediate and long-term effects of subchronic PQ-exposure differ. Also, PQ-exposure during this window of extensive neuronal differentiation led to a delayed microglial activation, of a character that could promote further pro-inflammatory signals that enable prolonged inflammation, thereby fueling further neurodegeneration.

© 2014 Elsevier Ireland Ltd. All rights reserved.

1. Introduction

Exposure to environmental toxicants is considered a risk factor in the etiology of neurodegenerative disease (Freire and Koifman, 2012; Wang et al., 2011). And exposure to the herbicide paraquat (PQ) has in epidemiological studies been connected to an increased risk of developing Parkinson's disease (Berry et al., 2010; Costello et al., 2009). PQ is one of the most widely used herbicides in the world, although its use is restricted in the US, and is banned in EU since 2007 (Cicchetti et al., 2009). PQ is transported into the brain by neutral amino acid transporters (Shimizu et al., 2001) and one known mechanism of toxicity is the induction of oxidative stress by producing a non-selective inhibition of all respiratory chain complexes in the mitochondria (Blesa et al., 2012; Gomez et al., 2007). In addition, PQ was shown to induce neuroinflammation

* Corresponding author at: Department of Physiology, University of Lausanne, 7, rue du Bugnon, CH-1005 Lausanne, Switzerland, Tel.: +41 21 692 55 21; fax: +41 21 692 55 05.
E-mail addresses: jenny.sandstrom@unil.ch, jsm.sandstrom@gmail.com (J. Sandström von Tobel).

0378-4274/$ – see front matter © 2014 Elsevier Ireland Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.toxlet.2014.02.001

both *in vitro* and *in vivo* (Mangano et al., 2012). Two hallmarks for neuroinflammation are the reactivity of microglial cells and astrocytes (Aschner, 1998; Streit et al., 2005). When activated, both cell types undergo changes in cell morphology and physiology, accompanied by increased expression/release of cytokines, chemokines, and stress proteins (Dong and Benveniste, 2001). Astroglial reactivity is mainly characterized by an increased expression of glial fibrillary acidic protein (GFAP) (Eng et al., 2000). Reactive microglial cells can assume either of two phenotypes: the alternative M2 phenotype, which favors tissue repair, and the M1 phenotype with neurodegeneration as an outcome (Kigerl et al., 2009; Perego et al., 2011). Microglial cells seem to play a central role in PQ neurotoxicity, since neuron–glia cultures depleted of microglial cells were resistant to low concentrations of PQ (Wu et al., 2005). However, in microglial cultures, PQ did not elicit a classical activation with increased cytokine expression (Klintworth et al., 2009), but it caused a dose-dependent increase in extracellular oxygen radicals (Wu et al., 2005), suggesting that NADPH oxidase 2 activation in microglial cells is essential for PQ neurotoxicity (Taetzsch and Block, 2013).

Landrigan et al. (2005) proposed that not only life long chronic exposure to low levels of neurotoxicants can lead to neurodegeneration, but that restricted exposure during developmental periods may condition the brain for developing a neurodegenerative disease later in life. An increasing body of evidence shows that neuroinflammation contributes to the progressive dopaminergic cell death observed in Parkinson's disease (Barnum and Tansey, 2010; Whitton, 2010). Therefore it is suggested that neuroinflammation elicited during an early developmental stage—a so-called window of vulnerability—could evolve into a detrimental character later in life. To investigate whether evidence to support this hypothesis could be found in an *in vitro* scenario, we applied low concentrations of PQ in 3D rat whole-brain aggregate cultures for 10 days during an early period of neuronal differentiation. Subsequently, PQ was washed out and, in order to observe possible long-term adverse effects, aggregates were cultured for another 20 days without further PQ-administration. During the time in culture neurons, astro- and microglia fully differentiate and evolve into a histotypic tissue, with cell–cell interactions that are imperative for the development of an inflammatory response (Monnet-Tschudi et al., 2007; Zurich et al., 2002). Adverse effects of PQ were observed immediately following the exposure and after the 20-day washout period. At both timepoints a proteomic approach was used for a global perspective on PQ-effects, and morphological assessments, as well as gene expression was implemented to examine effects on neurons and on glial reactivity, with an emphasis on determining M1 and/or M2 microglial activation.

2. Materials and methods

2.1. Chemicals and solutions

Paraquat dichloride (Sigma-Aldrich, 36541) was diluted in double-distilled water in stock solutions of 10, 50 and 100 mM concentrations. For exposure of the aggregate cell cultures aliquots of the stock solution were added directly to the culture medium in a 1:100 dilution. At each culture media change paraquat dichloride was added to maintain a constant concentration.

2.2. Cell culturing and experimental protocols

Aggregating brain cell cultures were prepared from whole fetal brains of embryonal day 16 Sprague Dawley rats (Janvier, France), and cultivated in chemically defined medium, as previously described in detail (Honegger et al., 2011). In short, whole brains were mechanically dissociated and cell suspension was after washing cultivated in flasks under constant gyratory conditions at a density of 6×10^7 cells per flask, resulting in spontaneous aggregate formation. The day of culture preparation is designated day *in vitro* 0 (DIV). Cultures were exposed to paraquat dichloride from DIV 5 to 15 at the concentrations of 0.1, 0.5 and 1 mM. Samples were collected at DIV15, directly after the 10-day exposure period. For observation of long-term effects, PQ was washed out at DIV15 and samples were collected at DIV 35 after a 20-day period without further toxicant administration.

2.3. Protein content and enzymatic activity measurements

Aggregates were washed in phosphate-buffered saline and homogenized by sonication—with 40% intensity and six repetitions of 3 s on, 2 s off intervals—in 400 ml of 2 mM potassium phosphate buffer containing 1 mM EDTA (pH 6.8). Homogenates were divided in aliquots for determination of total protein content, intracellular lactate dehydrogenase activity (LDH), glutamine synthetase (GS), choline acetyltransferase (ChAT) and glutamic acid decarboxylase (GAD) activities.

The Lowry method (Lowry et al., 1951) was used to determine protein content. Intracellular LDH (EC 1.1.1.27) was determined by conventional photometric assay as described by Koh and Choi (1987).

Intracellular GS activity (EC 6.3.1.2) was measured with a modified Pishak and Phillips method, using L-[1-^{14}C] glutamic acid as substrate and phosphoenolpyruvate/pyruvate kinase as the ATP-regenerating system (Patel et al., 1982; Pishak and Phillips, 1979).

Intracellular ChAT activity was measured according to the radiochemical method of Schrier and Shuster (1967) with modifications introduced by Wilson et al. (1972), measuring the conversion of radioactive acetyl-CoA and choline iodine into radioactive acetylcholine.

Intracellular GAD activity (EC 14.1.1.15) was determined according to the protocol of Wingo and Awapara (1950) measuring the conversion of radio active labeled glutamic acid by glutamic acid decarboxylase.

2.4. Gene expression analysis by semi-quantitative real time PCR

Total mRNA was prepared with the RNeasy kit (Qiagen, Switzerland) according to manufacturer's guidance and yield was determined by spectrophotometry (NanoDrop, ND-1000). Reverse transcription was performed on 1 mg total RNA with the High Capacity cDNA Reverse Transcription kit (Life Technologies, US). Real-time PCR analyses were performed using Power SYBR Green (Life Technologies, USA) and 3.2 ng of cDNA per reaction for detection of *Nefh* (fw 5′-caggacctgctcaacgtcaa-3′, rev 5′-cttcgccttccaggagttttct-3′), *Gfap* (fw 5′-ccttgacctgcgacttgag-3′, rev 5′-gcgcatttgcctctcacacaga-3′). Taqman gene expression assay (Life Technologies, USA) and a total of 50 ng cDNA per reaction were used to detect expression of *Il-4* (Rn01456865_m1), *Il-1β* (Rn00580432_m1) *Il-6* (Rn99999011_m1), *Tnf-α* (Rn99999017_m1), *Arg1* (Rn00691090_m1), *Mrc1*/*Cd206* (Rn01487342_m1), *Itgam*/*Cd11b* (Rn00709342_m1) and *Cd86*/B7-2 (Rn00571654_m1). Beta actin (Actb) was used as an internal control gene for both SYBR Green and Taqman detection and the ΔΔCt method (Livak and Schmittgen, 2001) was used to calculate relative mRNA expression.

2.5. Proteomic analysis

Aggregates were lysed in urea 6 M, dithiothreitol (DTE) 1 mM, triethylammonium bicarbonate buffer (TEAB) 0.1 M at pH 8 with hydrochloric acid (HCl) with four cycles of 7 s sonication (UI250v Vial Twitter, Hielsher, Teltow, Germany) and 1 min on ice between each cycle. After centrifugation at 4 °C at 20,800 rcf for 20 min, the supernatants were recovered and protein concentrations were determined by Bradford assay (Biorad, Hercules, USA). Replicate samples were pooled before proceeding to protein digestion with trypsin (DIV15; control and 0.5 mM PQ, DIV35; control and 0.5 mM PQ). An equal amount of proteins (36 μg) were used for each condition. The volume was normalized to 33 μl. Disulfide bonds were reduced with 2 μl of *tris*(2-carboxyethyl)phosphine (TCEP) 50 mM at 37 °C for 1 h and cysteines were blocked by alkylation with 1 μl iodoacetamide (IAA) at 400 mM. Urea concentration was lowered to < 2 M by addition of 67 μl TEAB 0.1 M before addition of trypsin (Sequencing Grade Modified Trypsin, Promega, USA). Tryptic digestion was performed overnight at 37 °C, using a ratio of enzyme to proteins of 1–50. Each peptide mixture was labeled with a different isobaric tag (TMT-6plex, Thermofisher, USA) according to manufacturer's protocol. After labeling, the peptides were separated in 24 fractions according to their isoelectric point using the 3100 OFFGEL fractionator (Agilent, USA) following manufacturer's protocol, desalted on microspin C18 columns (Harvard Apparatus, Holliston, USA), evaporated and solubilized in 5% AcN–0.1% FA prior to mass spectrometry analysis.

2.6. Quantitative protein content measurement by Western blotting

Aggregates were homogenized by sonication in lysis buffer containing 20 mM HEPES pH 7.5, 1.5 mM $MgCl_2$, 0.2 mM EDTA, 100 mM NaCl, 1× complete protease inhibitor cocktail (Roche, Switzerland). Protein content was measured at UV 280 nm (NanoDrop, Thermo Scientific). Fifty micrograms of total protein mixed 1:6 with Laemmli buffer (375 mM Tris–HCl pH 6.8, 9% SDS, 50% Glycerol, 9% Betamercaptoethanol, 0.03% Bromophenol blue) was separated on 10% SDS Page gels and transferred to polyvinylidene difluoride membranes (BioRad, Switzerland). Following blocking in 10% non-fat dry milk/20 mM Trizma base, 137 mM NaCl, 0.05% Tween, pH 7.6, membranes were incubated with primary antibody (GFAP, mouse monoclonal, 1:800, Sigma; NF-H/M/L, mouse monoclonal, 1:800, Enzo Lifesciences; TH, rabbit polyclonal, Millipore, 1:1000 and SYP, mouse monoclonal, Millipore,

Please cite this article in press as: Sandström von Tobel, J., et al., Immediate and delayed effects of subchronic Paraquat exposure during an early differentiation stage in 3D-rat brain cell cultures. Toxicol. Lett. (2014), http://dx.doi.org/10.1016/j.toxlet.2014.02.001

Download English Version:

https://daneshyari.com/en/article/5860165

Download Persian Version:

https://daneshyari.com/article/5860165

Daneshyari.com