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Docosahexaenoic acid and tetracyclines as promising neuroprotective compounds with poly(ADP-ribose) polymerase inhibitory activities for oxidative/genotoxic stress treatment ☆

Magdalena Cieslik *, Joanna Pyszko, Joanna B. Strosznajder

Medical Research Center, Polish Academy of Sciences, Department of Cellular Signaling, Pawinskiego 5 Str., 02-106 Warsaw, Poland

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ABSTRACT

The human genome is exposed to oxidative/genotoxic stress by several endogenous and exogenous compounds. These events evoke DNA damage and activate poly(ADP-ribose) polymerase-1 (PARP-1), the key enzyme involved in DNA repair. The massive stress and over-activation of this DNA-bound enzyme can be responsible for an energy crisis and neuronal death. The last data indicated that product of PARP-1, i.e. poly(ADP-ribose) (PAR), acts as a signalling molecule and plays a significant role in nucleus-mitochondria cross-talk. PAR translocated to the mitochondria can be involved in mitochondrial permeability, the release of an apoptosis-inducing factor (AIF). Its translocation into the nucleus leads to chromatin condensation, fragmentation and cell death. The exact mechanism of this novel death pathway has not yet fully been understood.

In this study the relationship between AIF and PARP/PAR in death signalling in the neuronal cell line (HT22) subjected to oxidative/genotoxic stress evoked by *N*-methyl-*N*'-nitro-*N*-nitrosoguanidine (MNNG) was explored. The neuroprotective influence of docosahexaenoic acid (DHA), major dietary ω-3 long-chain polyunsaturated fatty acids as well as the action of tetracyclines, the novel suppressors of PARP-1, were examined. The effect of these all compounds was compared with specific PARP-1 inhibitors.

The oxidative/genotoxic stress evoked by MNNG enhanced the level of PAR in a time-dependent manner with a concomitant significant decrease in the mitochondrial AIF protein level. Moreover, the down-regulation of the anti-apoptotic proteins (Bcl-2 and Bcl-xL) and the up-regulation of the Bax pro-apoptotic protein were presented. In these conditions massive HT22 cell death was observed. Both PARP-1 inhibitors: 3-aminobenzamide (3-AB) and PJ 34, tetracycline: doxocycline and minocycline, as well as DHA protected the cells against PAR formation and AIF translocation. Moreover, all of these compounds enhanced Bcl-xL gene expression and protected the cells against MNNG-induced death.

Our data show that both DHA and tetracyclines offer a novel neuroprotective strategy for oxidative/genotoxic stress treatment.

1. Introduction

The ability of cells to maintain genomic integrity is vital for cell survival and proliferation. Our genome is exposed to potentially deleterious oxidative/genotoxic events during every cell division cycle. The endogenous source of DNA damage results from the cellular metabolism or routine errors in DNA replication and recombination. In addition, cellular and organismal exposure to exogenous genotoxic agents, such as ultraviolet light, oxidative stress and chemical mutagens, leads to a variety of nucleotide modifications and DNA strand breaks. The chemical mutagen, *N*-methyl-*N*'-nitro-*N*-nitrosoguanidine (MNNG) is widespread in the environment (Chen et al., 2011). For many years it has been used as a direct-acting methylating agent. The effect of MNNG action is the production of a wide spectrum of DNA damage, including *O*^{6}*-alkylguanine* (*O*^{6}*AlkG*) (Kumaresan et al., 1995; Margison et al., 2002). The *O*^{6}*AlkG*DNA lesion is known to be cytotoxic, mutagenic and carcinogenic (Hour et al., 1999; Memisoglu and Samson, 1996) and can lead to programmed cell death. Moreover, MNNG can release nitric oxide (NO) and can contribute to free radical cascade activation. Protein nitration, which indicated peroxynitrite formation after MNNG treatment, was also observed (Bai et al., 2007; Pacher et al., 2007; Chiu et al., 2011).

Both oxidative and genotoxic stress can lead to DNA damage and to activation of the nuclear enzyme polymerase poly(ADP-ribose)

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* Corresponding author. Tel.: +48 22 6086420; fax: +48 22 6685223.
E-mail address: mcieslik@imdik.pan.pl (M. Cieslik).

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(PARP-1) (Romert and Jenssen, 1987). PARP-1 plays a key role in DNA repair *via* the base-excision-repair (BER) and nucleotide-excision-repair (NER) complex. PARP-1 is a highly conserved constitutively-expressed 116 kDa protein which belongs to the large family of PARP proteins sharing homology on the C-terminus catalytic domain and playing a critical role in gene transcription, cell cycle progression, chromatin function and genomic stability (Hassa and Hottiger, 2008; Strosznajder et al., 2010, 2011; Czapski et al., 2013). PARP-1 is responsible for 90% of poly(ADP-ribosylation) processes occurring in the central nervous system (Naegeli et al., 1989; Strosznajder et al., 2005, 2010; Abd Elmageed et al., 2011). In response to DNA damage, PARP-1 activity is rapidly increased, thus leading to the formation of long-chain poly(ADP-ribose) (PAR) (Ame et al., 2004; Kim et al., 2005; Schreiber et al., 2006). Overactivation of the enzyme under different stress conditions leads to the depletion of its substrate, βNAD^+, and then ATP. The excessive activation of PARP-1 has previously been proposed as being crucial to neuronal death in brain ischemia, inflammation and several other pathological conditions (Strosznajder et al., 2003, 2005; Virág, 2005; Moroni, 2008; Moroni et al., 2012) *via* a number of proposed mechanisms. The signalling event downstream of PARP-1 activation has not yet fully been identified. During excessive oxidative stress this enzyme, through auto-ribosylation, could be inactivated (Ueda et al., 1982). The last data show that the enzymatic product of PARP-1, i.e. PAR, can play a significant and complex role in cell death signalling. It has been proposed that PAR can be translocated to the mitochondria, where it may induce the release of the apoptosis-inducing factor (AIF), its translocation into the nucleus and, in consequence, cell death (Andrabi et al., 2006; Strosznajder and Gajkowska, 2006; Yu et al., 2006; Heeres and Hergenrother, 2007; Krantic et al., 2007; Strosznajder et al., 2010, 2011, 2012; Delavallée et al., 2011). The exact mechanism of this novel pathway as well as the overall role of PAR in cell death does not seem to be fully understood. However, modulation of the PAR level could be a therapeutic goal for cell death regulation. This can be done by inhibition of PARP-1 and modulation of poly(ADP-ribose) glycohydrolase (PARG), a key enzyme responsible for PAR degradation (Heeres and Hergenrother, 2007; Virág and Szabó, 2002). The inhibition of PARP-1 may protect cells against PAR-mediated AIF release.

In our studies we analysed the potential neuroprotective properties of PARP-1 inhibitors, docosahexaenoic acid (DHA), minocycline and doxycycline. DHA, a major dietary ω-3 long-chain polyunsaturated fatty acid (LCPUFA), is highly present in phosphatidylethanolamine in the brain and is a precursor of neuroprotectin NPD1 (Mukherjee et al., 2004; Bazan, 2005, 2009) which, down-regulates apoptosis and promotes cell survival (Serhan et al., 2006; Mukherjee et al., 2004, 2007). DHA is highly enriched in fatty fish oils and is synthesised *via* α-linolenic acid elongation and desaturation (Trumbo et al., 2002; Bazan, 2009). DHA is esterified into phospholipids and secreted as lipoproteins (Bazan, 2003), and is needed for the maintenance of neuronal and photoreceptor functions. Under normal, homoeostatic conditions DHA is esterified at the sn-2 position of membrane phospholipids as a free fatty acid and is present at a very low or undetectable level in the central nervous system. The amount of free DHA is rapidly increased under excessive oxidative stress in several pathological conditions (Bazan, 2003; Lukiw and Bazan, 2008). Clinical studies indicate that a low plasma DHA concentration is correlated with a number of brain diseases and with cognitive and behavioural defects during development and aging (Conquer et al., 2000; Noaghiul and Hibbeln, 2003). The potential benefit was shown by the supplementation of DHA in age-related cognitive decline (Jiang et al., 2009; Yurko-Mauro et al., 2010). A number of studies have also reported the beneficial effects of dietary DHA supplementation on cognition and synaptic integrity in AD models (Oster and Pillot, 2010).

The neuroprotective effect was also described previously for minocycline and other tetracycline derivatives, such as doxycycline (Yrjänheikki et al., 1998). Minocycline attenuated neuronal death after excitotoxicity in the cell culture and also in the animal models of stroke (Morimoto et al., 2005; Fox et al., 2005), ischemia (Song et al., 2006; Chu et al., 2010), Parkinson's disease (Wu et al., 2002; Du et al., 2001) and amyotrophic lateral sclerosis (Zhu et al., 2002). As shown by Alano et al. (2006), minocycline inhibits PARP-1 activation induced by genotoxic stress. It has been suggested that minocycline and also doxycycline have other targets besides PAPR-1. These tetracyclines could exert an anti-inflammatory and anti-oxidative effect (Rotem-Dai et al., 2009). However, the exact mechanisms of cytoprotection exerted by these compounds are not fully understood.

In this study we investigated the relationship between AIF and PARP-1 in mouse hippocampal HT22 cells in response to oxidative/genotoxic stress induced by MNNG. The response of pro-apoptotic and anti-apoptotic gene expression under genotoxic stress and its correlation to the survival of neuronal HT22 cells was analysed. Moreover, the cytoprotective effect of both DHA and tetracyclines in this stress condition was evaluated and compared with the effect of specific PARP-1 inhibitors.

2. Materials and methods

2.1. Materials

ECL reagents anti-rabbit IgG were purchased from Amersham Biosciences; Hoechst 33342 was from Riedel-de-Haën Germany; anti-mouse IgG was purchased from GE Healthcare, Piscataway NJ, USA. Anti-β-actin IgG was from MP Biomedicals, Irvine CA, USA; anti-AIF was from Santa Cruz Biotechnology CA, USA; MDL28170 was from Merck; High Capacity cDNA Reverse Transcription Kit and TaqMan Gene Expression Assays were from Applied Biosystems, Foster City, CA, USA; KT5823 was from Cayman Chemicals; GF109203x was from Enzo Life Sciences; anti-PAR antibody and anti-PARP-1 were from Alexis Corp.; other biochemical and inorganic reagents were from Sigma–Aldrich, St. Louis MO, USA.

2.2. Cell culture

The immortalized murine hippocampal cells (cell line HT22), was a generous gift from Prof. David Schubert (The Salk Institute, La Jolla, CA). HT22 cells were cultured in Dulbecco's modified Eagle's medium supplemented with 10% fetal calf serum (FCS), 50 U/ml penicillin, 50 μg/ml streptomycin and 2 mM glutamine at 37 °C in a humidified incubator containing 5% CO_2. Cells were subcultured about twice a week. For experiment, confluent cells were subcultured into polyethylenimine-coated 100 mm^2 dishes or 24-well plates. Cells were used for experiments at 75–90% confluence or one day after being plated in the 24-well plate. Prior to treatment, cells were replenished with 2% FBS medium or with serum-free medium.

2.3. Cells treatment protocols

HT22 cells were treated with *N*-methyl-*N*'-nitro-*N*-nitrosoguanidine (MNNG) at 50 to 500 μM concentration for various times up to 24 h. In some experiments, cells treated with MNNG were cultured for 48 h with 100 μM MNNG and the following inhibitors: PJ 34, 20 μM; 3-Amino Benzamide, 5 mM; Z-DEVD-FMK, 40 μM; α-Pifitrin, 20 μM; cyclosporine A, 2 μM; MDL, 10 μM; doxycycline, 2,5–20 μM; minocycline, 10–200 nM; docosahexaenoic acid,