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SUMMARY

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The role of apoptosis inducing factor (AIF) in amyloid β toxicity and in genotoxic stress evoked by *N*-methyl-*N*′-nitro-*N*-nitrosoguanidyne (MNNG)

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Warsaw 2012

Oxidative stress is considered as one of the main events in the pathogenesis of neurodegenerative disorders, including Alzheimer's and Parkinson's diseases. Despite intensive research, the exact mechanism leading to oxidative stress in these diseases is not precisely understood. Neither molecular phenomena nor cell death pathways caused by activation of oxidative and genotoxic stress are fully explained. The activity of free radicals may lead to changes in intracellular signal transduction, in the expression of genes, structural and functional changes, including the alteration of mitochondrial function and DNA repair. These changes may consequently lead to the cells death. Exploring mechanisms that are responsible for cell survival or death under different stress condition is important in the context of novel neuroprotection strategy.

Mitochondrial dysfunctions are key phenomena in the pathomechanism of many neurodegenerative disorders. Apoptosis inducing factor (AIF) is one of the key proteins involved in proper mitochondrial function. Poly(ADP-ribose) synthetase (PARS-1) is the nuclear recipient of free radical signaling and is a key enzyme in DNA repair, mitochondrial energy state and AIF level.

AIF is a mitochondrial intermembrane flavoprotein that belongs to a large family of proteins together with AMID and AIFL. Their physio-pathological functions are not yet fully understood. In physiological conditions AIF has the properties of NADH oxidoreductase, plays an important role in the oxidative phosphorylation and in the free radical defense (Susin et al., 1999; Miramar et al., 2001; Hangen et al., 2010). AIF contains three functional domains: FAD binding domain, NADH binding domain and C-terminal domain (Otera et al., 2005; Modjtahedi et al., 2006). The first two AIF domains are responsible for oxidoreductase activity and in physiological conditions are involved in the transport of electrons as the components of the complex I and III of the electron transport chain (Miramar et al., 2001; Vashen et al., 2004; Modjtahedi et al., 2006; Hangen et al., 2010). The C-terminal domain is responsible for the AIF apoptotic function. In response to the pro-apoptotic signal AIF is released from mitochondria and is translocated to the nucleus, where together with the cyclophilin A, induces chromatin condensation and DNA fragmentation, leading to the caspase-independent apoptosis (Hong et al., 2004; Yu et al., 2006; Krantic et al., 2007).

Previous studies have shown that AIF is a highly conserved protein that is essential for the embryonic development (Brown et al., 2006). Its absence leads to neurodegeneration (cerebellar atrophy, ataxia, blindness) (Klein et al., 2002). Moreover, low expression of AIF affects free radical homeostasis (Apostolova et al., 2006), which consequently leads to oxidative stress and to macromolecular damage. The oxidation or single- and double-strand breakage of DNA causes activation of the nuclear enzyme PARS, previously known as a polymerase (PARP) (Yu et al., 2002; Strosznajder et al., 2005, 2011; Moroni et al., 2011). PARS-1 is mostly sensitive sensor of DNA damage and key enzyme in chromatin modification and transcription regulation. PARS-1 plays an essential role in many physiological and pathological processes such as maintenance of genome stability, aging, inflammation or brain ischemia-reperfusion injury (Strosznajder et al., 2005, 2011; Beneke and Burkle, 2007, Moroni, 2008; Moroni and al., 2011). Under massive oxidative or genotoxic stress conditions, an excessive activation of PARS-1 as well as consumption of βNAD^+ and subsequently ATP take place. In addition, a product of PARS-1 enzymatic activity, poly(ADP-ribose) PAR, a new signaling molecule, can lead to AIF release from mitochondrial and its translocation to the nucleus (Yu et al., 2006). This result in caspase-independent apoptosis, known as parthanatos.

The aim of the present study was to elucidate the AIF gene expression and protein level in cell survival and death processes under amyloid β (Aβ) peptide toxicity conditions. The factors involved in the activation of oxidative/ nitrosative and genotoxic stress were included in the study. Moreover, the relationship between AIF level in mitochondria, PARS-1 activity/PAR level and cells survival was also examined. The cytoprotective effect of selected inhibitors and pharmacologically active compounds was estimated.

The studies were carried on the rat Pheochromocytoma PC12 cell lines and on immortalized murine hippocampal (HT22) cell lines. The PC12 cells were transfected with wild type human amyloid precursor protein gene (APPwt) or carried a double Swedish mutation (APPsw) as a model of $A\beta_{1-40}$ toxicity liberated endogenously. Moreover PC12 control cells were treated with exogenous Aβ peptide and subjected to nitrosative stress. The part of results about AIF level and expression after Aβ treatment were compared with data obtained in animal studies of transgenic mice sharing London mutation in APP gene (FVBTg $(Thy1;APP)^{Val717Ile}$) which represent the model of

Alzheimer's disease. In the study of the molecular phenomena affecting the mitochondrial level of AIF and its translocation to the nucleus, particular attention was paid to the activity and the integrity of nuclear enzyme PARS-1. The free radical level was measured by DCF fluorimetric test and the cells survival were estimated using the MTT test. The role of AIF in the molecular mechanisms of cells survival and death was also tested in genotoxic stress conditions induced by DNA alkylation evoked by MNNG.

Genotoxic agents are widely disseminated in the environment. These include components of cigarettes smoke, by-products of industrial processes or drugs used in anti-cancer therapy. The mechanism of action of these agents and the effects which are induced in living organisms, in particular in the nervous tissue, are not fully understood. Special attention was given to the role of PARS-1, the action of its inhibitors and also numerous other selected neuroprotective compounds were tested.

The presented research demonstrated an increase of mitochondrial AIF level in three PC12 cell lines transfected with human wild-type APP (APPwt) or APP carrying the double Swedish mutation (APPsw). Data demonstrated the relationship between AIF level, oxidative stress conditions and Aβ concentration in PC12 cells treated with exogenous $A\beta_{1-42}$. The nitrosative stress evoked by NO donor also induces enhancement of mitochondria AIF level in PC12 cells. In these conditions a small percentage of cell death was observed. Moreover, the enhanced AIF level was shown in cortex and striatum in $APP^{+/+}$ transgenic mice, an animal model of Alzheimer's disease (AD). In these experimental conditions a significant reduction in PARS-1 activity in APPsw cells and after NO donor treatment was demonstrated.

The release of the AIF from mitochondria to the nucleus was found in genotoxic stress conditions caused by the DNA alkylating agent MNNG. Concomitantly the accumulation of PAR, that indicated enhancement of PARS-1 activity, was observed. These changes accompanied a significant pool of cell death. Under these experimental conditions of PARS-1 inhibitors (PJ 34 and 3-AB), tetracycline of third generation (mino- and doxycycline) and docosahexaenoic acid (DHA) significantly protected the cells against death caused by MNNG. Another important result showed the inhibition of calpain (protein probably involved in the AIF release) and ERK1/2 kinase, both of which demonstrated a positive effect on cell viability in these stress conditions.

Moreover, our data demonstrated that the genotoxic stress evoked by DNA alkylation significantly decreased antiapoptotic Bcl2 and BclXl gene expression and increased proapoptotic Bax gene expression.

In conclusion, the obtained data indicate a significant role of mitochondrial AIF protein level in the defence/adaptive processes (enabling the PC12 cells survival) in the conditions of Aβ toxicity and in the presence of NO donor, SNP. The reduced PARS-1 activity observed may be related to the presence of NO released by Aβ and/or to enzyme auto-modification. It may also be the degradation of PARS-1 as a result of the caspase-3 activation. The reduced PARS-1 activity excludes the participation of enzymes in the death mechanism, but may have significant importance in the disruption of DNA repair processes.

Our data indicate that PARP/PAR/AIF signaling pathway is responsible for hippocampal HT22 cell death evoked by genotoxic stress. The inhibitors of PARP-1, DHA and tetracyclines protect mitochondrial integrity and function, and also protect the cells against death. Moreover, the inhibition of calpain and ERK1/2 revealed additional beneficial effect. PKC, PKG, p53 and caspase-3 are not engaged in cells death evoked by MNNG

The obtained results can help understand the cell death mechanism in oxidative and genotoxic stress conditions that accompany neurodegenerative diseases and exposition to numerous environmental toxins. In addition the results can facilitate the identification of effective cytoprotection.