Adaptive anti-oxidative responses to chronic exposure to stress-signaling molecule, oxidized cell-free DNA, in rat neural cells

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Abstract. Stress is an adaptation reaction to harmful environment changes. Both oxidized and non-oxidized cell-free DNA (oxo-cfDNA and no-cfDNA) liberating from damaged cells possesses a characteristic of stress-molecules. CfDNA molecules modified by oxidation exhibit more oxidative (increasing 8-oxodG in DNA) and antioxidative (enhanced transcription of NRF2 and NRF2-dependent genes) activities than no-cfDNA in non-neural cell cultures. Whether the oxo-cfDNA molecules affect brain cells under different regimens of treatment that may mimic multiple, chronically applied stressful insults remained unclear. We studied if multiple stimulations of nervous system cells with oxidized cfDNA may further augment the DNA oxidative damage by inducing 8-oxodG modifications, and increase antioxidant Hmox1 gene expression and the NRF2 protein level in a primary cell culture of rat cerebellum (RCC). We found that daily treatment of RCC with oxo-cfDNA for 3 days increased accumulation of 8-oxodG in intracellular DNA, activated transcription of Hmox1 gene and enhanced the NRF2 protein level. However, after eleven-day daily treatment, the opposite effect was observed, in which 8-oxodG level in intracellular DNA and Hmox1 gene expression and NRF2 protein level in cerebellar cells were significantly decreased. Therefore, under subchronic, 3-day regimen of treatment, oxidized cfDNA further augmented accumulation of 8-oxodG in cellular DNA and promoted activation of the antioxidant system whereas its 11-day chronic action decreased both activities presumably due to inducing tolerance to stress-signaling via overactivation and/or exhaustion of molecular targets of oxo-cfDNA in cerebellar cells.

1. Introduction
External damaging effects of environmental factors may be stress development cause [1-4]. Stress activates the oxygen radical’s generation (ROS), and subsequently oxidative stress development [5]. DNA molecules are susceptible to ROS that results in accumulation of oxidized nucleosides in the cells. Most commonly, the ROS effect is 8-oxodG formation in both intracellular and cfDNA [6-7]. It has
been shown that oxo-cfDNA is a biologically active molecule, which activates the ROS synthesis and promotes antioxidant and cytoprotective processes in cell populations [7-8, 20].

The antioxidant system, including NRF2, leads to a decrease in the DNA oxidative modifications. However, excessive activation of defence mechanisms causes an opposite effect. Thus, the OGG1 enzyme is capable to excise 8-oxodG from DNA, whereas excessive OGG1 activation is the cause of chromatin hydrolysis and cell death [9].

Previously, we showed that the short-term RCC treatment with oxo-cfDNA promoted activation of brain cell defence systems during the first day after nucleic acid addition: Nrf2 and Hmox1 genes expression increased and the NRF2 protein translocated to the cell nuclei [18]. The study also demonstrated that cfDNA concentration and the level of its 8-oxodG raised in subchronic (2-3 days) and prolonged (11 days) stress in vivo. Simultaneously, long-term stress, according to the Hans Selye concept, can lead to pathological processes. [10-11]. It can be assumed that prolonged oxo-cfDNA as a stress-molecule action, might have an adverse effect on RCC. That assumption is based on the fact that an increase in the cfDNA concentration has been detected in various pathological conditions and was associated with a poor outcome [12-18]. However, it is still unclear whether the oxo-cfDNA plays a damaging or protective role. Therefore, that study aims to evaluate the effects of the prolonged (3-11 days) oxidized cfDNA effects on RCC.

2. Materials and method
RCC were prepared according to the protocol described previously [18]. Briefly, the cerebellum was lysed (trypsin-Versene-EDTA solution) and cells were placed on 6-well Nuclon™ plates (Thermo Fisher Scientific, USA). After three days of RCC stabilization, the oxo-cfDNA was added into the cultivation medium daily for 3 and 11 days (50 ng/ml). Purification and oxidation of rat brains DNA to be employed in RCC treatment was carried out according to the procedure described previously [18]. Briefly, DNA of newborn rat brain tissue was isolated and oxidized by 3% H2O2 solution with UV exposure (λ = 312 nm, 90 sec). After RCC incubation with the oxo-cfDNA, Hmox1 gene transcriptional activity (housekeeping gene was Tbp) was analysed by real-time PCR on a StepOne device (ABS, USA) and molecular markers were visualised (NRF2, 8-oxodG) by fluorescence microscopy (Zeiss, Germany). For microscopy the RCC were fixed with a 4% formaldehyde solution and perforated with a 0.5% Triton solution in PBS. For the molecular marker analysis, primary anti-8-oxodG antibodies (Santa Cruz Biotechnology, USA), primary anti-NRF2 antibodies (Bioss, USA) and FITC conjugated antibodies (Santa Cruz Biotechnology, USA) were used.

3. Results and discussion

3.1. The level of 8-oxodG after 3-11-day daily oxo-cfDNA RCC exposure
Daily oxo-cfDNA treatment of RCC for three days resulted in no alteration in the amount of 8-oxodG compared to the control (Figure 1). After eleven-day daily oxo-cfDNA RCC exposure to the 8-oxodG level in the culture was significantly reduced (figure 2).

3.2. NRF2 protein level after 3-11-day daily oxo-cfDNA RCC exposure
Three-day daily oxo-cfDNA RCC exposure results in NRF2 protein content elevation and migration mainly in RCC nuclei (Figure 3). Eleven-day daily oxo-cfDNA exposure the level of NRF2 leads to NRF2 protein amount reduction (figure 4).

3.3. Hmox1 gene transcription
Heme oxygenase 1 (HMOX1) is the main brain antioxidant protein supporting the normal mitochondria and ROS functions and synthesizing carbon monoxide (II) [19]. The Hmox1 transcription level in RCC increased 7.6-fold, but decreased 3.6-fold after three- and eleven-day daily oxo-cfDNA exposure respectively (figure 5a, b).
Figure 1. Fluorescence microscopy: 8-oxodG (FITC). 3-day oxo-cfDNA exposure.

Figure 2. Fluorescence microscopy: 8-oxodG (FITC). 11-day oxo-cfDNA exposure.

Figure 3. Fluorescence microscopy: NRF2 protein (FITC). 3-day oxo-cfDNA exposure.

Figure 4. Fluorescence microscopy: NRF2 protein (FITC). 11-day oxo-cfDNA exposure.

Figure 5. RT-PCR: Hmox1 gene expression after 3-11-day daily oxo-cfDNA exposure.
Note. *p < 0.01, Mann-Whitney Rank Sum Test. Power of performed test with alpha = 0.050: > 0.9.
3. Conclusions

Subchronic daily oxo-cfDNA exposure RCC exposure for three days increased pro- and anti-oxidative processes as revealed by Hmox1 gene transcription and the NRF2 protein level. Contrariwise, prolonged daily oxo-cfDNA RCC exposure for 11 days significantly decreased oxidative DNA damage, Hmox1 gene expression and the NRF2 protein level. We propose that the effect of chronic exposure of brain cells to oxidized DNA is presumably due to the tolerance induction to stress-signaling via overactivation and/or exhaustion of oxo-cfDNA targets in the nervous system cells. Data may explain the development neurological abnormalities during continuous stressful conditions.

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