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Age-dependent guanine oxidation in DNA of different brain regions of Wistar rats and prematurely aging OXYS rats

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ABSTRACT

Background: Oxidative damage to the cell, including the formation of 8-oxoG, has been regarded as a significant factor in carcinogenesis and aging. An inbred prematurely aging rat strain (OXYS) is characterized by high sensitivity to oxidative stress, lipid peroxidation, protein oxidation, DNA rearrangements, and pathological conditions paralleling several human degenerative diseases including learning and memory deterioration.
Methods: We have used monoclonal antibodies against a common pre-mutagenic base lesion 8-oxoguanine (8-oxoG) and 8-oxoguanine DNA glycosylase (OGG1) in combination with indirect immunofluorescence microscopy and image analysis to follow the relative amounts and distribution of 8-oxoG and OGG1 in various cells of different brain regions from OXYS and control Wistar rats.
Results: It was shown that 8-oxoG increased with age in mature neurons, nestin- and glial fibrillary acidic protein (GFAP)-positive cells of hippocampus and frontal cortex in both strains of rats, with OXYS rats always displaying statistically significantly higher levels of oxidative DNA damage than Wistar rats. The relative content of 8-oxoG and OGG1 in nestin- and GFAP-positive cells was higher than in mature neurons in both Wistar and OXYS rats. However, there was no significant interstrain difference in the content of OGG1 for all types of cells and brain regions analyzed, and no difference in the relative content of 8-oxoG between different brain regions.
Conclusions: Oxidation of guanine may play an important role in the development of age-associated decrease in memory and learning capability of OXYS rats.
General significance: The findings are important for validation of the OXYS rat strain as a model of mammalian aging.

1. Introduction

Partially reduced oxygen species are produced as intermediates and by-products of aerobic respiration and generated in cells through exposure to ionizing radiation and other agents. These reactive species, including $^{\bullet}O_2^-$, H_2O_2, and $HO^{\bullet}$, are potent oxidants attacking many cellular components including mitochondrial and nuclear DNA [1–4]. Oxidative stress can induce premutagenic DNA base lesions, such as 8-oxoguanine (8-oxoG), thymine glycols, formamidopyrimidine (imidazole ring-opened) derivatives of guanine and adenine, etc. 8-oxoG is one of the most common oxidatively damaged DNA bases [5]. This lesion is mutagenic, resulting in G→T transversions [6,7].

Oxidative damage to the cell, including the formation of 8-oxoG, is ongoing and has been regarded as a significant factor in carcinogenesis and aging [2–4,8,9]. Inherited overproduction of free radicals in mammals is accompanied by a number of pathologies resembling human degenerative diseases such as cataracts, cardiomyopathy, emphysema, carcinogenesis, and others, as well as short life-span and low fertility [10–13]. Normally, oxidative lesions are removed from DNA by specific enzymes, DNA glycosylases [14]. Human [15], mouse [16], and rat [17,18] 8-oxoguanine-DNA glycosylases (OGG1) removing 8-oxoG from DNA have been cloned and characterized. This enzyme has two catalytic activities, acting as a DNA glycosylase that removes the 8-oxoG base and then as an AP lyase cleaving the DNA backbone at the resulting abasic site through β-elimination. Yet, despite the existence of repair mechanisms, an imbalance between formation and repair of DNA lesions including 8-oxoG leads to an accumulation of DNA damage with age [19].

The studies of mechanisms of oxidative stress-dependent pathologies greatly benefit from the availability of animal models with increased sensitivity to free radicals. A number of animal models have

Abbreviations: Abs, antibodies; DAPI, 4′,6-diamidino-2-phenylindole; GFAP, glial fibrillary acidic protein; OGG1, 8-oxoguanine DNA glycosylase; 8-oxoG, 8-oxoguanine; PBS, phosphate-buffered saline

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been successfully used in the studies of aging and age-related disorders [11,12,20–22]. However, there are only a few examples of genetic models with inherited features of accelerated aging. For example, the strain of senescence-accelerated mice (SAM) represents a widely used model of accelerated aging [11,12,21]. SAMP8 mice are considered an animal model with increased sensitivity to free radicals possessing a defective energy transfer mechanism that suggests a dysfunction of the normal cell metabolism [11,12,21]. These mice demonstrate early development of learning and memory deterioration concomitant with a decrease in the expression of a 8-oxo-dGTPase MTH2 and an increase in the level of 8-oxoG in the hippocampus in comparison with a control line, beginning at ~5 months of age [23]. Mice deficient in an ERCC1–XPF endonuclease involved in nucleotide excision DNA repair also demonstrate an accelerated aging phenotype and accumulate oxidative lesions, such as 8,5′-cyclopurines, in their DNA [24,25].

The OXYS rat strain was bred from the Wistar stock by selection for the susceptibility to a cataractogenic effect of a galactose-rich diet and mating of highly susceptible siblings [26]. First it was shown that the main characteristics of this inbred strain are increased sensitivity to oxidative stress, high level of lipid peroxidation and protein oxidation, DNA rearrangements, and cataract formation [10]. Dietary antioxidants significantly decrease the level of protein oxidation and lipid peroxidation in OXYS rats and protect the animals from cataract formation [27–29].

An immunofluorescence assay was applied for determination of 8-oxoG in DNA isolated from different organs of rats [30]. It was shown that for both OXYS and Wistar rats, the 8-oxoG content in lung DNA is 1.3–2.0-fold higher compared with liver DNA. The amount of 8-oxoG in liver and lung DNA of the OXYS rats increases with age and is 1.5–2.4-fold higher than in DNA of Wistar rats. In a later study, monoclonal Abs against 8-oxoG in combination with indirect immunofluorescence microscopy and image analysis were used to follow the relative age-dependent amounts and distribution of 8-oxoG in the liver tissue from OXYS and Wistar rats [31]. 8-OxoG increases with age in both strains, with OXYS rats always displaying significantly higher levels of oxidative DNA damage than Wistar rats.

A large amounts of experimental data accumulated over the past decade demonstrate that the OXYS strain can be an appropriate model of accelerated senescence [32–39]. Recently, it was shown that senescence-accelerated OXYS rats are suitable for studies of age-related cerebral dysfunction. The behavior of young OXYS rats is similar to the behavior of old Wistar rats. The specific behavioral alterations in OXYS rats such as high anxiety and low exploratory activity do not appear at birth but rather develop at 4–12 weeks of age [40]. As a result, at the age of 3–4 months, OXYS rats exhibit a significantly reduced locomotor and exploratory activity in the open field test and the hole-board task, increased anxiety in the elevated plus maze test, and abnormal associative learning in the passive avoidance task [34,35,41,42]. Using magnetic resonance imaging, we have recently detected the first signs of neurodegeneration (diffusion changes) in OXYS rat brain at the age of 3 months, and by 12 months it progressed to focal changes detected mainly in the cortex and the anterior horns of the lateral ventricles [39]. In addition, OXYS rats show an early development of age-associated pathological phenotypes similar to several geriatric disorders observed in humans, including cataract and retinopathy [37,38]. It was hypothesized that the accelerated senescence of OXYS rats is also associated with progressive mitochondrial dysfunction and, indeed, dietary supplementation with antioxidants can prevent the premature deterioration of mitochondrial function typical of OXYS rats [10,27,36].

The frontal cortex is the most differentiated section of the nervous system, ultimately coordinating all processes of a mammalian organism including the highest functions (memory, learning, consciousness, etc.). The hippocampus is one of the most structured and highly organized regions of the brain participating in memory consolidation, spatial coding, and formation of emotions [43]. Morphologically well-distinguished features of the hippocampus include the CA-1 and CA-3 fields and the dentate gyrus, each of which consists of a number of strata harboring axons or cell bodies. For example, stratum pyramidale in the CA-1 and CA-3 fields contains large bodies of pyramidal neurons, while stratum granulosum in the dentate gyrus is formed by small bodies of granular cells.

Brain may be exposed to elevated oxidative stress due to its high metabolic rate [19,44]. Age-associated oxidative DNA damage accumulates in the cells of both cerebral cortex [9] and hippocampus [45]. The increase in the rate of lipid peroxidation is one of the earliest events in the development of Alzheimer's disease [46,47]. Therefore, study of these brain regions is important for understanding the debilitating consequences of oxidative damage. Recently, CA1 and CA3 fields have been shown to play a critical role for rapid contextual learning and memory recall [48], the processes that seem to be disturbed in OXYS rats.

Since OXYS rats are characterized by early-onset defects in learning, memory, and behavior, this strain could be exploited as a model to study mechanisms of the development of brain dysfunction with age. As a step towards validation of this model, in the present work we have estimated the levels of 8-oxoG and OGG1 in frontal cortex and CA1 and CA3 hippocampal fields of OXYS and Wistar rats, the regions that control many neural functions apparently affected in OXYS rats. The age of the animals, 1 to 3 months, was that at which the behavioral deviations of OXYS rats become apparent.

2. Materials and methods

2.1. Materials, chemicals, and animals

Reagents used in this work were obtained from Merck and Sigma-Aldrich. Aqueous buffers and working solutions were treated with Chelex 100 resin (Bio-Rad Laboratories, Hercules, CA) to remove trace transition metals and degassed to minimize their soluble oxygen content. We have used monoclonal N45.1 mouse antibodies (Abs) to 8-oxoG (JaICA, Japan), rabbit polyclonal Abs to OGG1 (Abcam, Cambridge, MA), goat Abs to nestin (Santa Cruz Biotechnology, Santa Cruz, CA), chicken Abs to glial fibrillary acidic protein (GFAP; Millipore, Billerica, MA), FITC-conjugated donkey anti-mouse Abs, Texas Red-conjugated donkey anti-rabbit Abs, Cy3-conjugated donkey anti-chicken Abs, and Cy3-conjugated donkey anti-goat Abs (Jackson ImmunoResearch Laboratories, West Grove, PA).

Male 1- and 3-month-old Wistar and OXYS rats [26] used in this study were housed at the Institute of Cytology and Genetics animal breeding facility in breeding colonies under standard conditions and handled according to the institutional ethical guidelines. The number of animals included in the study was five for 1-month-old Wistar rats and six for all other groups (3-month-old Wistar, 1-month-old OXYS and 3-month-old OXYS).

2.2. Preparation of sections for microscopy

The organ sampling protocol conformed to the local animal ethics committee guidelines. After general anesthesia and decapitation, the brain vasculature was perfused with 100 ml of 0.2 M phosphate buffer (pH 7.4). Then the brain was fixed using 100 ml of 0.02 M phosphate buffer (pH 7.4) containing 4% paraformaldehyde. The skull was opened and the brain was additionally fixed with 100 ml of PBS (2 mM KH_2PO_4, 10 mM Na_2HPO_4, 3 mM KCl, 137 mM NaCl; pH 7.4) containing 4% paraformaldehyde for 4 h at 4 °C. The brain was washed three times with PBS (20 min each) at room temperature and placed for 24 h to PBS containing 20% sucrose at 4 °C and then for another 24 h to PBS containing 30% sucrose at 4 °C. After the cryoprotection, the brain was frozen in hexane at −40 °C and stored at −80 °C. A set of frozen sagittal microscopic cryosections of different brain regions (18 μm thick) was obtained using an HM 505 N cryostated microtome (Thermo Fisher Scientific, Walldorf, Germany).