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Age-dependent increase of etheno-DNA-adducts in liver and brain of ROS overproducing OXYS rats

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Abstract

Reactive oxygen species (ROS) and lipid peroxidation (LPO) play a role in aging and degenerative diseases. To correlate oxidative stress and LPO-derived DNA damage, we determined etheno-DNA-adducts in liver and brain from ROS overproducing OXYS rats in comparison with age-matched Wistar rats. Liver DNA samples from 3- and 15-month-old OXYS and Wistar rats were analyzed for 1,N^6-ethenodeoxyadenosine (εdA) and 3,N^4-ethenodeoxycytidine (εdC) by immunoaffinity/^{32}P-postlabelling. While εdA and εdC levels were not different in young rats, adduct levels were significantly higher in old OXYS rats when compared to old Wistar or young OXYS rats. Frozen rat brain sections were analyzed for εdA by immunostaining of nuclei. Brains from old OXYS rats accumulated εdA more frequently than age-matched Wistar rats. Our results demonstrate increased LPO-induced DNA damage in organs of OXYS rats which correlates with their known shorter life-span and elevated frequency of chronic degenerative diseases.

Keywords: Etheno-DNA-adducts; Lipid peroxidation; OXYS rats; Aging; Degenerative diseases

Inherited overgeneration of free radicals in experimental animals is accompanied by pathologic conditions resembling human degenerative diseases of aging such as cataracts, cardiomyopathy, emphysema, scoliosis, carcinogenesis, loss of the long associative memory as well as a shortened life-span [1–4]. By selection from Wistar stock for the susceptibility to a cataractogenic effect of galactose-rich diet and siblings mating of highly susceptible rats, a unique inbred strain of rats (OXYS) was developed in the Institute of Cytology and Genetics of Russian Academy of Science (Novosibirsk, Russia) [5]. The main characteristics of the OXYS strain are inherited ROS overproduction, high level of lipid peroxidation, protein oxidation, DNA rearrangements, and morbid conditions mimicking the above-mentioned human degenerative diseases [6]. Thus, the OXYS strain offers a good model to study how etiopathogenesis is related to oxidative DNA-damage, LPO, and DNA-repair [7]. Our previous studies suggested that the promutagenic, chemically stable etheno-(ε-) DNA-adducts appear to be useful markers for assessing oxidative stress and LPO-derived DNA damage in early stages of carcinogenesis [8] and that such DNA damage could play a major role in the development of human cancers especially those that have an inflammatory component in their etiopathogenesis [9]. In the present work, we have applied our sensitive and specific methods for the determination of etheno-DNA-adduct levels in liver and brain from OXYS and age-matched Wistar rats. Marked strain differences and age-associated changes in DNA-damage were observed in liver and brain of ROS overproducing OXYS rats.

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Materials and methods

Animals. Male white Wistar and OXYS rats from the Institute of Cytology and Genetics of Russian Academy of Sciences (Novosibirsk, Russia) [5] at 3 and 15 months of age were used in this study. The animals were housed in the colonies under standard conditions. The rats were decapitated, the livers and brain were immediately removed and frozen in liquid nitrogen.

DNA-adduct analysis in liver. DNA was extracted from isolated nuclei using Qiagen columns according to the manufacturer's protocol with minor modifications (pH of the elution buffer was set to 7.4 and NaCl concentration was increased to 1.4 M). εdA and εdC were analyzed in liver DNA of OXYS and Wistar rats by an immunoaffinity/^{32}P-postlabelling method [10]. In brief, 25 μg of DNA was hydrolyzed to nucleotide-3′-monophosphate, using micrococcal nuclease and spleen phosphodiesterase. Normal nucleotides were quantitated by HPLC, and the adducts were enriched on immunoaffinity columns prepared from the monoclonal antibodies EM-A-1 for εdA and EM-C-1 for εdC. The antibodies were obtained through a collaborative study with M. Rajewsky (University of Essen, Essen, Germany). The adducts and the internal standard deoxyuridine-3′-monophosphate were labelled with [γ-^{32}P]ATP (>5000 Ci/mmol) and T4-polynucleotide kinase [CAUTION: [γ-^{32}P]ATP is a hazardous radioactive compound and should be handled with sufficient protection and shielding]. The adducts are resolved on polyethyleneimine-TLC plates, using two-directional chromatography, and the relative adduct levels per parent nucleotides were calculated. Our method has a detection limit of about five adducts per 10^{10} parent nucleotides, which permits their measurement in small amounts of DNA.

Immunohistochemical detection of εdA in tissue sections. Frozen rat brain sections were analyzed for εdA at cellular levels by immunohistochemistry, using a method developed in our laboratory [11] with modification that Mab EM-A-1 was used instead of EM-A-4. In brief, frozen liver tissue sections were fixed with acetone on a slide and air-dried. Endogenous peroxidase activity was quenched, and histone and non-histone proteins and RNA were removed from the brain sections by treatment with Proteinase K and with RNase. Non-specific binding sites were blocked with BSA. To denature DNA, sections were treated with HCl for 10 min, rinsed with water, and the pH was neutralized. The sections were further incubated with the purified primary Mab for εdA (EM-A-1) at 4 °C overnight. The washed sections were incubated with the biotinylated second goat anti-mouse antibody and with the avidin-biotin-peroxidase complex. To visualize the reaction, 3,3′-diaminobenzidine was used as a chromogen. After stopping the reaction in PBS, slides were mounted with Kaiser's glycerine gelatine. The intensity of staining was evaluated on photomicrographs obtained with a Leitz Laborllux 11 microscope equipped with a color camera.

Statistical analysis. Student's *t* test was used for comparisons of adduct levels in liver tissues.

Results and discussion

LPO of ω-6 polyunsaturated fatty acids, such as linoleic and arachidonic acids, induced by ROS results in DNA-reactive aldehydes such as *trans*-4-hydroxy-2-nonenal (HNE), malonaldehyde, and crotonaldehyde, which are increasingly implicated in carcinogenesis. These intermediates can react with DNA bases to form exocyclic DNA adducts of which several have been characterized as propano- and etheno-(ε)-DNA-base adducts [12,13]. Of the latter 1,N^6-ethenodeoxyadenosine (εdA), 3,N^4-ethenocytidine (εdC), and N^2,3-ethenodeoxyguanosine have been detected in vivo (Fig. 1). These promutagenic, chemically stable secondary DNA-oxidation products appear to be useful markers for assessing oxidative stress-derived DNA damage in chronic degenerative diseases [14]. DNA samples from four to five rats aged 3 and 15 months of the OXYS and Wistar strain were analyzed for εdA and εdC (chemical structures, see Fig. 1) by an immunoaffinity/^{32}P-postlabelling method after hydrolyzing to 3′-monophosphates, purification by immunoaffinity chromatography and subsequent ^{32}P-labelling to 5′-monophosphates and TLC [10]. The ε-adduct levels were not different in liver DNA of 3-month-old OXYS rats when compared to Wistar rats of similar age (mean εdA/10^9 dA: 6.3 vs. 5.8; εdC/10^9 dC: 29.1 vs. 40.1) (Fig. 2). However, both adduct levels were significantly higher (Student's *t* test) in 15-month-old OXYS rats when compared to Wistar rats of similar age and to 3-month-old OXYS rats (mean εdA/10^9 dA: 38.5 vs. 4.5; εdC/10^9 dC: 97.5 vs. 20.4). These results clearly demonstrate that in the liver of aging OXYS rats, oxidative stress and LPO are induced, causing the formation of promutagenic ε-DNA-adducts. An age-dependent increase in hepatic

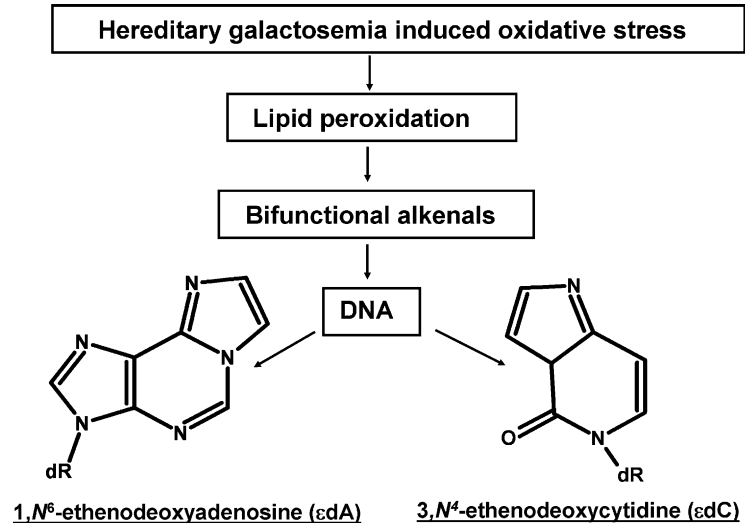

Fig. 1. Possible pathway for the formation of etheno-DNA-adducts (εdA, εdC) via LPO in OXYS rats.