Characteristics of the Liver Microsomal Membranes in Mice with Genetically Determined Accelerated Aging

M. O. Yuneva, E. M. Aleksandrova, E. G. Kurella*, and A. A. Boldyrev*

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The liver membranes of SAMP mice with accelerated aging display increased inducibility of peroxide processes at early stages of life (2 months) and an initially higher level of MDA at later stages (8 months). In comparison with the liver membranes of the control SAMR strain, liver membranes of rapidly aging animals are characterized by more dense packing (higher microviscosity), increasing with age, judging from the eximerization of the membrane-binding fluorescent probe pyrene. Therefore, the differences in LPO and membranous bilayer packing occur in SAMP mice at early stages of development (earlier than the neurological parameters) and contribute to specific functioning of membrane-associated enzymes.

Key Words: *age-specific changes; SAM mice; lipid peroxidation; pyrene eximerization*

As neurodegenerative hereditary diseases, age-associated changes in the brain are paralleled by increased production of free-radical compounds [7]. The relationships between these processes are little known. It is presumed that accumulation of free radicals in tissues, which drastically increases in disease and with aging, is a cause of cell damage and dysfunction [5,7]. New strain of mice with accelerated aging (Senescence Accelerated Mice, SAM), has been widely used for investigation of age-related changes in tissues [6]. With aging, the deterioration of the neurological parameters in the brain of these mice is paralleled by an increase in the production of free radicals and a decrease in antioxidant defense [6]. In order to find out whether these regularities can be extrapolated to other tissues, we investigated the age-associated changes in the liver microsomal membranes of SAMP mice (prone to age-associated degenerations). The reference strain was SAMR (mice relatively resistant to these processes).

International Biotechnology Center, M. V. Lomonosov Moscow State University; *Laboratory of Clinical Neurochemistry, Institute of Neurology, Russian Academy of Medical Sciences, Moscow

MATERIALS AND METHODS

SAMP and SAMR mice aged 2-9 months were used. The animals were maintained under standard vivarium conditions. After sacrifice, the liver was perfused with cold 100 mM K-phosphate buffer (pH 7.4), homogenized on the cold, and the microsomes were isolated by the Haiosh method [2]. Isolated fractions were stored at -20°C for no longer than 2 weeks.

The initial level of LPO products (MDA) was evaluated in the TBA test; their accumulation was measured at various intervals after addition of $FeSO_4$ and ascorbate to the final concentrations of 10 and 200 μM [4]. MDA accumulation was determined at the site of the maximum peroxide process (on the 90th min of incubation) and denoted as LPO inducibility in the studied membranes. Microviscosity of membranes was evaluated by the ratio of the eximer/monomer pyrene fluorescence [3]. Pyrene eximerization was measured in a Hitachi F3000 spectrofluorimeter at 25°C in the presence of 300 μg protein (1-ml sample, stimulation at 330 nm, extinction at 395 nm (monomer) and 470 nm (eximer). The results were processed routinely using Student's test.

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RESULTS

Morphological signs of accelerated aging are detected in SAMP mice after 4 months of life, after which they progress far more rapidly than in SAMR mice [6]. During the same period, intense production of free radicals in brain tissue is observed.

The initial level of TBA-reactive products (MDA) in the liver microsome membranes of SAMP mice markedly increased between the age of 2 and 8 months (Fig. 1). In SAMR mice the level of MDA in the liver microsomes did not increase with age.

The induction of MDA accumulation in the presence of iron and ascorbate helps estimate how much biological material in a sample can be oxidized and how rapidly this process develops. The maximum level of MDA in all experiments was attained in 90 min, varying in membrane preparations from mice of different strains (Fig. 2). The membranes of young SAMP animals (2 months) were more prone to LPO than those of SAMR mice. With age these differences leveled, indicating alteration of the lipid composition of microsomal membranes.

Our findings indicate that the level of LPO substrates in the liver membranes of SAMP mice is higher than in SAMR mice. More intense oxidation of these compounds in SAMP mice leads to the leveling of LPO inducibility with age and to a drastic rise of peroxide content in the membranes from SAMP mice.

Different concentrations of oxidation substrates in the liver membranes of the studied mouse strains correlated with the compactness of their packing, as evaluated by pyrene fluorescence (Fig. 3). In SAMP mice, pyrene eximerization decreased (increase in the bilayer microviscosity) with age. Moreover, studies of the pyrene eximerization showed that the membrane structure is less developed in SAMR mice than in age-matched SAMP mice. Based on these data, we have concluded that the differences in the lipid composition of liver membranes in SAMP mice manifest themselves at the earliest stages of life (when there are no morphological differences) and are apparently genetically programmed. These differences should influence the functioning of membrane-associated enzymes, which deserve special investigation.

Thus, in SAMP mice the pathological changes involve both brain and liver membranes, and we have demonstrated a similar pattern of damage in them. The membrane defects manifest themselves in a higher microviscosity (compactness of packing) of the bilayer; this suggests that a higher level of free radical production during aging is caused by dysfunction of integral enzymes induced by alterations of the cell membrane structure [6].

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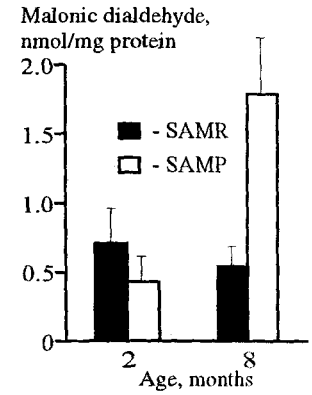

Fig. 1. Initial level of LPO products in microsomal preparations of the liver of SAMR and SAMP mice. Here and in Fig. 2: protein concentrations in a sample 1 mg/ml.

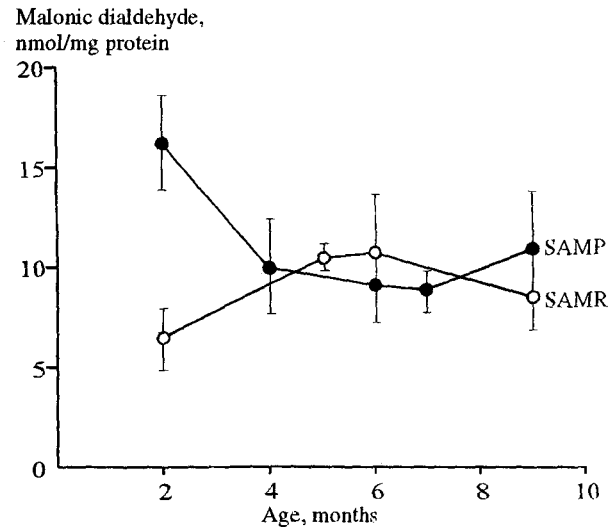

Fig. 2. Age-related changes in LPO inducibility in liver microsomal of SAMR and SAMP mice by the 90th min of LPO induction by iron and ascorbate.

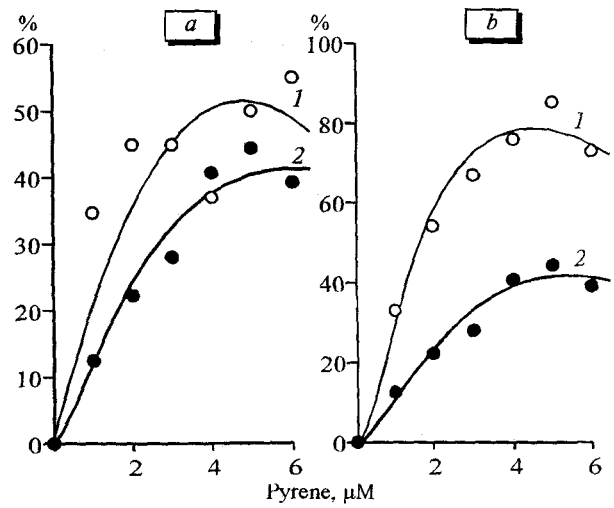

Fig. 3. Pyrene eximerization (%) in liver microsome preparations. *a*) SAMP mice aged 2 (*1*) and 8 (*2*) months; *b*) 8-month-old SAMR (*1*) and SAMP (*2*) mice.

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