Deficits in learning ability and aging of skin in both strains of senescence-accelerated mouse (SAM) P8 and P10: neuropathological, neurochemical, histological and pharmacological analysis

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Animal models of age-related deficiencies are required to elucidate the fundamental mechanisms of age-related deficiencies of learning or aging of skin, the establishment of pertinent animal models that have characteristics very similar to those of human dysfunctions is needed to develop prophylactics/therapeutics against age-related disease. The SAM (senescence-accelerated mouse) has been established as a murine model of SAM strains, groups of related-inbred strains including nine strains of accelerated senescence-prone, short-lived mice (SAMP) and three strains of accelerated senescence-resistant, long-lived mice (SAMR). SAMP strain mice show relatively strain-specific age-associated phenotypic pathologies such as shortened life span and early manifestation of senescence. Among SAMP strain mice, SAMP8 and SAMP10 mice show an age-related deterioration in learning ability and skin aging. We review the neuropathological, neurochemical, histological and pharmacological features of SAM strains, particularly those of SAMP8 and SAMP10 strains, and the effects of several drugs on biochemical, behavioral and histological alterations in the SAMP8.

Key words senescence-accelerated mouse, onion, water maze, spatial learning, skin.

Introduction

The recent increase in the percentage of aged people in the population highlights the concern of age-associated diseases, particularly senile dementia and aging of skin. The establishment of pertinent animal models that have characteristics very similar to those in humans is essential in order to elucidate the fundamental mechanisms of age-related deficits and to develop effective drugs for the prevention of age-related diseases such as learning dysfunction and aging of skin. To address these problems, useful animal models of age-associated diseases need to be established. The senescence-accelerated mouse (SAM) was established as a murine model of accelerated aging by Takeda *et al.*[1] In the present study, we characterized the neurochemical changes in the brain and skin aging of SAMP8 and 10 strains. We also demonstrated the beneficial effects of onion extract on learning deficits and skin aging in the SAM.

SAMP (prone) strains are unique and appropriate models for studies on aging because these strains have an "accelerated senescence" phenotype. In SAMP strains, normal development and maturity of reproductive function were observed, and the values of many (but not all) physiological and pathological parameters were found to be the same as those in normal-aging SAMR (resistant) strains at young to mature age. Prone strains of the SAM show shortened life span and early manifestation of senescence such as loss of activity, alopecia, lack of hair glossiness, skin coarseness, periophthalmic lesions, increased lordokyphosis and systemic senile amyloidosis (Table 1). These characteristic pathological phenotypes are similar to those often observed in elderly humans, which include senile osteoporosis, osteoarthritis, age-related deficits in learning and memory with/without forebrain atrophy, presbycusis, senile amyloidosis, and age-related impairment of immune response. The common aging characteristic of SAMP strains is senescence acceleration after normal development and maturation.

Learning and behavioral dysfunction in SAMP8 and SAMP10 strains

Among the SAMP strains of mice, SAMP8 mice have impaired acquisition and retention of passive avoidance response.[2] SAMP8 mice also show significant impairments in one-way active avoidance, T-maze active avoidance, and Sidman active avoidance, where the mice were trained to avoid foot shock. In spatial learning tasks, impairments in SAMP8 mice were observed in water-filled multiple T-maze and Morris's water maze tasks, in which the mice were trained to escape from the water.[2,3] SAMP8 mice also have age-related emotional disorders characterized by reduced anxiety-like behavior.[4]

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Table 1 Phenotypes and survival periods of SAM strains

	Phenotypes	Mean survival period (days)
SAMP1	Senile amyloidosis, Hearing impairment, Impaired immune response	297
SAMP2	Senile amyloidosis, Contracted kidney, Impaired immune response	277
SAMP3	Degenerative arthrosis of temporomandibular joint	415
SAMP6	Senile osteoporosis	243
SAMP7	Senile amyloidosis, Thymoma	269
SAMP8	Deficits in learning and memory	299
SAMP9	Cataract	273
SAMP10	Brain atrophy, Deficits in learning and memory	333
SAMP11	Senile amyloidosis, Contracted kidney	211
	SAMP combined	291
SAMR1	Normal senescence	568
SAMR4	Normal senescence	438
SAMR5	Normal senescence	462
	SAMR combined	489

Table 2 Neurochemical and histochemical alterations in the SAMP8 mouse brain in comparison to those in the SAMR1 mouse brain.

	SAMP8	Comments
NMDA receptor/channels	↓	Deficits in learning and memory
mACh receptors	↓	
Protein kinase C	↓	
Inflammatory cytokines	↑	
GDNF, NT3	↓	
5-HT$_{1A}$ receptors	CC ↑ HP ↓	Anxiety
Central BDZ receptors	CC ↑ HP ↓	
Anti-GFAP immunostaining	↑	Gliosis
Peripheral BDZ receptors	↑	
APP mRNA expression	↑	Aging
C-terminal fragment of APP	↑	

mACh: muscarinic acetylcholine, GFAP: glial fibrillary acidic protein, BDZ: benzodiazepine, APP: amyloid precursor protein, CC: cerebral cortex, HP: hippocampus, ↑: increase and ↓: decrease in SAMP8 vs SAMR1.

SAMP10 mice exhibited age-related brain atrophy and learning impairments in avoidance tasks.[5,6] SAMP10 mice also showed behavioral depression in a tail suspension test[3] and in a forced swimming test.[7]

Genetic and biological background of SAMP mice

The senescence-accelerated mouse (SAM) strains were established by Takeda (Kyoto University) in 1981.[1] The SAM strains were developed from the AKR/J strain. Since selective breeding was based on data of senescence, life span and pathologic phenotypes in addition to sufficient generations of sister-brother mating, they can be considered as inbred strains. This strain is a group of related-inbred strains that includes nine strains of accelerated senescence-prone, short-lived (SAMP) mice and three strains of accelerated senescence-resistant, long-lived (SAMR) mice. The mean life span in the P series was 9.7 months, 40% shorter than that in the R series (16.3 months) (Table 1). Data on biochemical and immunogenetic markers revealed that there was at least one genotype in each SAM strain that differed from that in the authentic AKR/J strain.[8] Furthermore, analysis of endogenous murine leukemia virus (MuLV) proviral markers showed there are several MuLV provirus markers found only in the SAMP strains or SAMR strains.[9] A comparison of the panels of proviral loci revealed that all of the SAM strains are genetically similar but are distinguishable from one another and that the SAM strains resemble but are clearly different from the parental AKR/J strain. These findings suggest that the SAM strains are a group of recombinant inbred strains or related-inbred strains developed by some accidental out-breeding between the AKR/J strain and one or more unknown strains.

Genetic analysis of learning and memory deficits in SAMP8 mice assessed by step-through passive avoidance tests and cross mating between SAMP8 and JF1 normal mice was performed. Results of the analysis show good agreement with Mendelian inheritance. Estimation of the number of genes using Wright's formula shows that at least two or three genes are involved in the development of learning and memory deficits in SAMP8 mice. Thus, learning and memory deficits in SAMP8 mice may be inherited by a relative small number of genes and regulated by a major single gene.[10] We further performed genetic analysis of SAMP8 mice using a whole genome scan for quantitative trait loci (QTLs) specifying the impairment of learning and memory deficits. Genetic markers were typed 113 loci spanning all chromosomes except the Y chromosome. Five loci with significant linkage to chromosomes 1, 12 and 13 have been identified.[11]

Neuropathological changes in the SAMP8 mouse brain

The SAMP8 mouse showed age-related appearances of spongy degeneration in the magnocellular reticular formation of the brain stem.[12] Electrolytic lesioning of this region of the brain stem induced severe deterioration.[13] The level of [^{3}H]PK-11195 (for ω3-benzodiazepine receptor), a marker of astroglial cells, was higher in brains of SAMP8 mice than in brains of SAMR1 mice, suggesting gliosis that may follow neuronal damage in the SAMP8 mouse brain.[14] This is supported by a marked immunoreactivity of

antibodies against glial fibrillay acidic proteins (GFAP, a marker protein of astrocytes) in SAMP8 brain. It has been reported that [^{3}H]PK-11195 binding is increased in the brains of patients with Alzheimer's disease. Immunoblot analysis revealed that reaction of a 27 kDa-protein with an antibody against the C-terminal peptide fragment of APP, but not reaction of 90-130-kDa APP (probably APP_{695}), was increased in the SAMP8 mouse brain, indicating abnormal formation of APP-like proteins in the SAMP8 mouse brain.[14] β/A4 protein-like immunoreactive granular structures were observed in various regions, including the medial septum, cerebral cortex, hippocampus, cerebellum, and some cranial nerve nuclei and roots, and increased markedly in number with aging/advance of age.[15] Chronic treatment of SAMP8 mice with Y-29794, a specific inhibitor of prolyl endopeptidase, reduced the number and density of Aβ-positive granular structures in the hippocampus.[16] In a quantitative immunohistochemical study, age-related degeneration of oligodendrocytes occurred in the hippocampus of SAMP8 mice.[17] SAMP10 mice exhibited age-related brain atrophy characterized by age-related loss and shrinkage of neurons in the cerebral neocortex and learning impairments in avoidance tasks.[5,6]

Neurochemical changes in the brains of SAMP8 and SAMP10 mice

The contents of glutamate (Glu) and glutamine (Gln) were higher in the brains of SAMP8 mice than in the brains of SAMR1 mice, while the contents of aspartate and alanine was lower, suggesting that a metabolic pathway from α-ketoglutarate to Glu predominates in the SAMP8 mouse brain.[17] Depolarization-stimuli evoked release of endogenous Glu and aspartate, and levels of non-neurotransmitters (Glu, alanine) were increased at 10 months of age in SAMP8 mice, suggesting fragility of nerve terminals of SAMP8 at old stages.[18] It is notable that local accumulation of excitatory amino acids induces neurotoxic effects. Cerebral cortical [^{3}H]MK-801 binding to N-methyl-D-aspartate (NMDA) receptor/channels in the SAMP8 mouse was lower than that in the SAMR1 mouse, indicating that NMDA receptor functions seem to be deficient in neurons of the SAMP8 mouse brain.[19] NMDA-induced release of [^{3}H]acetylcholine (ACh) and [^{3}H]noradrenaline (NA) was markedly reduced in SAMP8 mice.[20,21] Since NMDA receptor/channels are found on both soma and terminals of ACh- and NA-containing neurons, the reduction in [^{3}H]MK-801 binding as well as in NMDA-induced release of [^{3}H]ACh and [^{3}H]NA in the SAMP8 mouse brain strongly suggest functional deterioration of glutamatergic, cholinergic and noradrenergic neurotransmissions in the SAMP8 mouse brain.[20,21] Furthermore, [^{3}H]QNB binding and [^{3}H]pirenzepine binding activities of M_1 ACh receptors were decreased in the hippocampus, and the [^{3}H]rauwolscine binding (to α_2-adrenoceptors) was increased in the cerebral cortex. As has recently been shown, it is known that protein kinase C (PKC) and calmodulin-dependent protein kinase II (CaMKII) are key enzymes in long-term potentiation (LTP) in the hippocampus. The binding activities of [^{3}H]phorbol-12, 13-dibutyrate (PDBu) (for PKC) in both cytosol and membrane fractions in the hippocampus of aged SAMP8 mice were reduced.[19] Proinflammatory cytokines, such as IL-1β, IL-6 and TNFα formation of neuritic plaques in Alzheimer's disease[22] and in impairment in long-term potentiation,[23] or learning ability.[24] In the hippocampus of the SAMP8 mouse, the expression levels of IL-1β, IL-6 and TNFα mRNA were significantly elevated in comparison with those in the SAMR1 mouse.[25] These neurochemical alterations may also be related to dysfunction of learning and memory in SAMP8 mice.

The level of neurotrophin-3 (NT-3) mRNA in the cortex was higher in SAMP8 mice than in SAMR1 mice, whereas in the midbrain, hippocampus, and forebrain, NT-3 expression levels were lower in SAMP8 mice than in SAMR1 mice during the early development period.[26] GDNF mRNA expression levels in SAMP8 and SAMP10 mice was less than those in SAMR1 mice. Immunohistochemistry revealed that cells were positive for GDNF-like activity in SAMP8 and SAMP10 mice were diffusely distributed, in part around the pyramidal cell layer in the hippocampus.[27] Brain-derived neurotrophic factor mRNA levels in SAMP8 and SAMR1 mice were almost the same.[26]

The ligand binding activity of muscarinic ACh receptors decreased in the hippocampus of SAMP10 mice, and the protein kinase C level in the hippocampus of SAMP10 mice was lower than that in SAMR1 mice.[28] In the cortex of SAMP10 mice, an agonist binding of D_2/D_3 dopamine receptors increased significantly compared with that in SAMR1 mice. In the hippocampus of SAMP10 mice, an agonist binding of $5\text{-}HT_{1A}$ serotonin receptors increased.[7] These neurochemical alterations may also be related to dysfunction of learning and memory and depressive behavior in SAMP10 mice (Table 3).

In the brain, skin and liver of the SAM, increases in lipid peroxide[29] and protein oxidation, decreases in glutathione content[30] and in activity levels of superoxide dismutase (SOD)[30] and catalase,[31] and impairment in transport of Cu, Zn-SOD into mitochondria after cytosolic synthesis[32] were observed. Mitochondrial functions in the SAMP8 mouse such as respiratory control ratio, active uptake of calcium and the amount of Bcl-x were decreased.[33] Administration of a free radical scavenger prolonged the mean life span of the SAM.[34] As a cause of accelerated senescence, it has been postulated that an increase in genera-

Table 3 Neurochemical and histochemical alterations in the SAMP10 mouse brain in comparison to those in the SAMR1 mouse brain

	SAMP10	Comments
loss and shrinkage of neurons	CC	Brain atrophy
mACh receptors	↓	Deficit in learning and memory
Protein kinase C	↓	
$5\text{-}HT_{1A}$ receptors	HP ↑	Depressive behavior
D_2/D_3 dopamine receptors	CC ↑	

mACh: muscarinic acetylcholine, CC: cerebral cortex, HP: hippocampus, ↑: increase and ↓: decrease in SAMP8 vs SAMR1

tion of oxygen radicals or hypofunction of defense activity against oxidative stress may cause an accumulation of damage in proteins, lipids and nucleic acids.

Skin aging of SAMP10 mice

We also characterized the histological changes in the skin of SAMP mice as a model of chronoaging and photoaging of skin. In the dermis of SAMP10 mice, solar elastosis and an increase in elastin content, which are characteristic of human photoaging, were observed. The dermis of SAMP10 mice also shows increases in granular cell layer thickness, incidence of compact stratum orneum, glycosaminoglycans, inflammatory infiltrate and in number of mast cells, which are also characteristics of human photoaging. On the other hand, the dermis of SAMP10 mice shows decrease in hair follicles, increase in the number and the area of sebaceous glands and decrease in thickness of fatty tissue, which are characteristics of intrinsic human aged skin. Therefore, SAMP10 may be a useful animal model of human chronoaging and photoaging of skin.

Effects of several drugs on pathological alterations in SAMP8 mice

Several drugs improved the biochemical alterations and deficit in learning and memory. Long-term treatment with acidic fibroblast growth factor (aFGF), which has been shown to protect against neuronal death and affect neuronal differentiation, improved learning deficiency in SAMP8 mice and normalized the medial septohippocampal circuit, which is necessary for learning and memory.[35] Interestingly, aFGF also suppressed the reduction in cerebral cortical mACh- and NMDA-receptors and the immunohistochemical reduction in choline acetyltransferase in the septum of SAMP8 mice.[32] Subcutaneous administration of facteur thymique serique (FTS) enhanced the activities of Cu, Zn-SOD in the brain of SAMP8 mice brain, and decreased malondialdehyde (a lipid peroxide) content in the SAMP8 mice brain.[36] Administration of N-tert-butyl-α-phenylnitrone (PBN), a free radical scavenger, protected the cortical synaptosomal membrane protein structure in SAMP8 mice from oxidative stress.[37]

Long-term administration of Dan-Shen methanol extract (Salviae miltiorrhizae radix), which enhances blood flow in peripheral organs, improved spatial learning and emotional functions and increased the number of cerebral cortical NMDA receptors[38] (Table 4). The dietary α-linolenate/linoleate balance affects the n-3/n-6 ratio of brain phospholipid acyl chains and improved in the Sidman active avoidance task and in the light/dark discrimination task.[39] Onion extract and dipropyl-trisulfide, an organosulfur constituent of onion, improved the spatial learning ability of SAMP8 mice by means of Morris's water maze task. Moreover, SAMP8 mice given dipropyl-trisulfide showed a reduction in phosphatidylcholine hydroperoxide content, an indicator of hyperoxidative condition, in the hippocampus (unpublished observation). Treatment with onion extract improved characteristics of aged skin, i.e., prevention of increase in glycosaminoglycans and increase in the number of mast cells and inflammatory infiltrate.

Conclusion

We demonstrate the alterations in neurochemical and pathological parameters in SAMP8 and SAMP10 mice brain and skin. SAMP8 and SAMP10 mice seem to be useful animal models for evaluating drugs/foods intervening dementia, depression and aged skin.

Table 4 Effects of long-term DME or LSB treatment on various neurotransmitter systems in SAMP8 mice

DME	Cortex	Hippocampus	Striatum	Cerebellum	Brain stem
ChAT	-	-	-	-	-
QNB	-	-	-	-	-
MK-801	↑	-	-	-	-
NNA	-	-	-	-	↑
PDBu/m	-	↓	↓	↓	-
PDBu/s	-	-	↑	↑	-
LSB					
ChAT	-	-	↓	↓	-
QNB	-	-	-	-	-
MK-801	-	-	-	-	↓
NNA	-	-	-	↑	↓
PDBu/m	-	-	-	-	↓
PDBu/s	-	↑	-	-	↓

DME (500 mg/kg, p.o.) or LSB (60 mg/kg, p.o.) was administered for 3 weeks to SAMP8 mice. After behavioral experiments, neurochemical experiments were performed.
ChAT: choline-acetyltransferase, QNB : quinuclidinyl benzilate, MK-801: (+)-5-methyl-10,11-dihydro-5H-dibenzo[*a,b*]-cyclohepten-5,10-imine maleate, NNA: *N*-nitro-L-arginine, PDBu/m: PDBu binding in membrane fractions, PDBu/s: PDBu binding in soluble fractions.
↑: significant increase, ↓: significant decrease, - : no change.

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