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Carnosine, the Protective, Anti-aging Peptide

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Carnosine attenuates the development of senile features when used as a supplement to a standard diet of senescence accelerated mice (SAM). Its effect is apparent on physical and behavioral parameters and on average life span. Carnosine has a similar effect on mice of the control strain, but this is less pronounced due to the non-accelerated character of their senescence processes.

KEY WORDS: Carnosine; oxidative stress; aging; senescence accelerated mice (SAM).

ABBREVIATIONS: SAM Prone, SAMP–Senescence Accelerated Mice Prone; SAM Resistant, SAMR–Senescence Accelerated Mice Resistant, Ros–Reactive Oxygen Species.

INTRODUCTION

Carnosine (β-alanyl-histidine), a natural dipeptide characteristic of innervated tissues of vertebrates, was described as early as in 1900 [1]. The amount of carnosine in muscle and brain [2] exceeds that of ATP, but its biological functions are still obscure. Carnosine may serve as an effective pH buffer, providing about 60% of the proton-binding capacity in skeletal muscles [3, 4]; it is also an ion-chelating agent which neutralizes the damaging effect of ions of heavy metal such as copper, iron and cobalt [5]. In 1984, an antioxidant activity of carnosine was described when lipid peroxidation of biological membranes was stimulated *in vitro* [6]; this phenomenon was later confirmed by a number of other approaches [7–9].

The mechanism by which carnosine protects tissues against oxidative stress has been analyzed recently; carnosine may be a potent hydrophilic antioxidant directed mainly toward superoxide anion oxygen and hydroxide radical [10, 11]. The ability of carnosine to protect tissues against oxidative stress was also demonstrated in *in vivo* experiments under hypoxic and ischemic conditions [12, 13]. In addition to antiradical activity protecting membrane lipids and proteins from oxidative stress,

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carnosine has been found to possess anti-glycating properties which prevents the damaging effect of aldo- and keto-sugars on biomacromolecules during oxidative stress [14–16].

These properties of carnosine suggest that it might have anti-aging activity which could attenuate the development of senile features [17]. Here we have tested the anti-senescence effect of carnosine using mice with a genetic program of accelerated senescence (so-called Senescence Accelerated Mice, SAM). SAM are characterized by accelerated development of senile properties which are induced by increased generation of reactive oxygen species [18].

MATERIALS AND METHODS

Animals

In our experiments SAM-Prone (SAMP1 strain, 160 animals) and SAM-Resistant (SAMR1 strain, another 160 animals) mice of both sexes were used at ages ranging from 4 to 15 months, SAMR1 being used as the control for SAMP1 [19]. The animals were obtained from the Council for SAM Research (University of Kyoto, Japan). They were fed a standard commercially available diet and kept in an animal room with air conditioning at 25°C [19].

Consistent with data from the literature [17, 18], the first features of aging of SAMP1 mice appeared at the age of 4–5 months, and these features became pronounced by the age of 7–8 months. At the age of 6 months the first observations of mortality of SAMP1 animals were made. Mice of the control strain (SAMR1) at this age were characterized by normal behavioral and physical features.

Carnosine Treatment

To characterize the protective effect of carnosine against the development of senescent features, we randomly distributed animals of both strains into two subgroups of 80 mice each starting from the age of 4 months. For each strain one subgroup was fed the standard diet while the other was treated with carnosine which was added to drinking water in an amount corresponding to a daily dose of 100 mg/kg body weight, the dose effective in prevention of rat brain against hypoxia [13].

Statistics

The characteristics of the animals were monitored using the Grading Score System (GSS) [19]. Data were statistically analyzed using routine computer program. p values less than 0.05 were considered as statistically significant.

RESULTS

Physical characteristics and mortality of the animals were monitored throughout the experiment (from 4 to 16 months of age, when all the animals of the SAMP1 group had died). By the end of the experiment the death of a significant number of

Table 1. Morphological and Physiological Characteristics of 10-month old SAM under Different Conditions (Criteria used are from the GSS Representative Analysis; % of Animals in Groups Corresponding to Each Criterion is Presented; in Each Group $n = 36$)

Parameter	SAMR1	SAMP1	SAMP1 + Carnosine
1. Skin and hair:			
(a) loss of hair	0	91 ± 1#	92 ± 2
(b) loss of glossiness	0	95 ± 1#	56 ± 7***
(c) coarseness	0	73 ± 2#	78 ± 2
(d) skin ulcers	0	36 ± 3#	14 ± 2***
2. Eyes:			
(a) corneal opacity	0	48 ± 2#	45 ± 2
(b) periophthalmic lesions	0	92 ± 1#	78 ± 2***
3. Spinal lordokyphosis	6 ± 2	83 ± 3#	72 ± 3**
4. Physiological behavior:			
(a) reactivity	73 ± 7	9 ± 2#	58 ± 4***
(b) passive avoidance	67 ± 2	17 ± 2#	23 ± 1*
5. Body weight	30.7 ± 0.9	29.0 ± 0.6	28.0 ± 0.7

= $p < 0.001$ for SAMR1 vs. SAMP1; * = $p < 0.05$, ** = $p < 0.02$ and *** = $p < 0.01$ for SAMP1 vs. SAMP1 + carnosine.

SAMR1 animals had also occurred. The behavioral features and physical characteristics of the SAMP1 and SAMR1 animals are compared in Table 1; the data are in good agreement with the senescence-accelerated nature of the SAMP1 strain. The table shows that at 10 months of age SAMP1 animals were characterized by partial loss of hair (91% of animals), appearance of skin ulcers (36% of animals), periophthalmic lesions (92% of animals), and other physical features like lordokyphosis. Nearly all SAMP1 animals had decreased reactivity and only 17% demonstrated the normal reaction of passive avoidance. The main features of SAMR1 animals at 10 months of age were close to normal (non-accelerated) senescence as noted earlier [20]. The average body weight of mice of both strains remained at the same (expected) level.

No large differences were found in the behavioral characteristics of carnosine-treated and control SAMR1 animals. Their average body weights were 30.7 ± 0.9 g (control and 29.3 ± 0.5 g (carnosine-treated); these values are not statistically significantly different. Some differences were noted in physiological reactions: at 10 months of age only 27% of the control SAMR1 group had normal reactivity, while for the carnosine-treated group the corresponding value was 53%. Normal passive avoidance was observed for 33% of control and 41% of carnosine-treated mice ($p < 0.05$).

Consistent with the data in the literature, our data illustrate a significant difference in the average life spans of SAMP1 and SAMR1 animals and accelerated senescence of SAMP1 animals (Fig. 1).

Our data allow us to compare the effect of carnosine on the mortality curves of the two studied strains. The effect of carnosine is rather clear on comparison of the mortality curves presented in Fig. 1. Carnosine-treated SAMR1 animals were

characterized by slightly higher viability between 12 and 14 months of age; no difference was found at earlier and later ages.

The difference between the mortality curves of carnosine-treated and control SAMP1 animals was much more pronounced; it was statistically significant for the period between 8 and 15 months of age (Fig. 1). For the carnosine-treated SAMP1 animals maximal life span did not increase significantly, but the mean life span was 20% longer (calculations were done as in [19]) and the number of animals living to older ages was increased.

Differences in physical characteristics of both groups of mice were also pronounced; the differences for SAMP1 animals were more marked (Fig. 2). Morpho-physiological characteristics of these animals showed an apparent effect of carnosine inhibiting the accumulation of senile features (Table 1). For only a few parameters no difference was found between carnosine-treated and control SAMP1 animals, for example, loss of hair and coarseness of skin. However, for some other parameters a marked protective effect of carnosine against aging was observed, e.g., for periophthalmic lesions ($p < 0.001$), physiological reactivity ($p < 0.001$), skin ulcers ($p < 0.001$) and spinal lordokyphosis ($p < 0.02$). According to the GSS parameters, the carnosine-treated animals can be characterized as more resistant to the development of features of aging. However, the body weight of animals of both groups did not change, so the effect of carnosine was not connected with the activation of anabolic processes.

DISCUSSION

One of the causes of accelerated senescence in SAMP1 animals is known to be a deficit in the antioxidant defense system which is accompanied by an over-production of reactive oxygen species (ROS) in their tissues [18, 20, 21]. These data suggest that ROS scavengers might be protective. Recently, data have been published showing that 14-day injection of the synthetic ROS scavenger *N*-tert-butyl-α-phenylnitrone at dose 30 mg/kg body weight resulted in pronounced protection of brain membranes against oxidative attack [22].

However, synthetic ROS scavengers may not be suitable for clinical use because of their apparent negative side effects during systemic treatment. Thus, a number of natural antioxidants and membrane protectors, such as tocopherols, ascorbic acid, and carotenes have been suggested as food additives to treat patients under chronic ROS attack [23]. Carnosine might be also suitable for use as a natural protector [5, 13, 24].

In addition to the data already presented here, some biochemical characteristics of brain and other tissues of SAM have been studied. They clearly demonstrate that metabolic features of SAMP1 are actually connected with over-production of ROS [20], and carnosine may serve as a potent natural defense against intracellularly generated ROS [13].

The biological mechanism of the effects of dietary carnosine remains unclear. In rodents, carnosine taken with food penetrates mainly (by 80%, see Ref. [5]) into the blood stream and because of relatively low activity of serum carnosinase is only

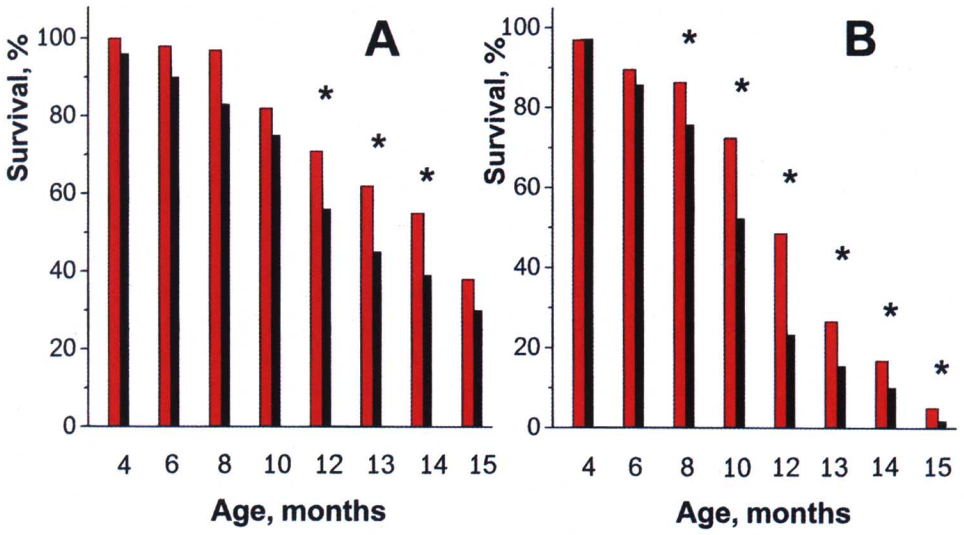

Fig. 1. Survival of SAMR1 (A) and SAMP1 (B) animals under different conditions. Each sub-group analyzed began as 80 animals at 4 month age. Asterisks above bars correspond to difference between carnosine treated (red bars) and untreated (black bars) sub-groups which is statistically significant at $p < 0.05$.

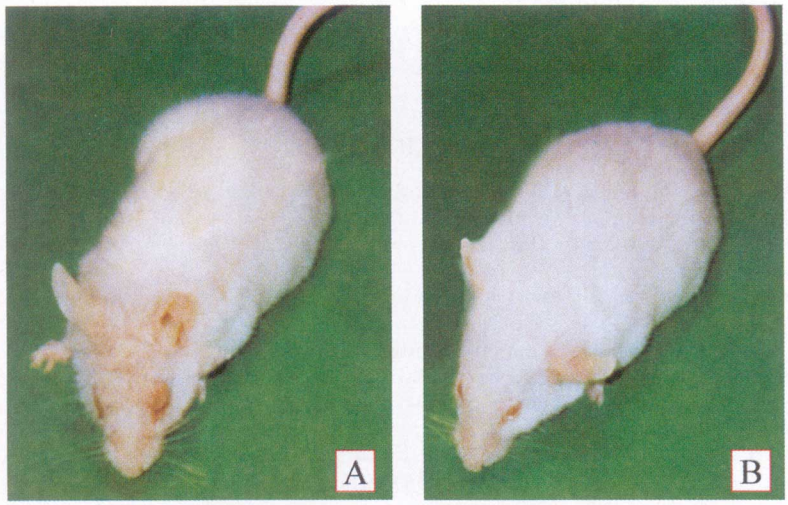

Fig. 2. Physical features of 9 month old SAMP1 animals with no (A) and with (B) carnosine treatment.

partially split before being accumulated by the cells. Thus, as a result of such treatment carnosine may directly affect metabolic pathways by suppressing the cellular level of ROS because of the useful properties discussed above. This reasonable suggestion is supported by the presence of special carnosine transporters facilitating its penetration through the brush border or blood-brain barrier and causing carnosine accumulating within cells [5, 25]. Secondary effects of products of carnosine metabilism cannot be totally excluded, but the expected products of carnosine hydrolysis, β-alanine and histidine, do not have such protective properties, and histidine even degrades the behavioral features of animals [26].

As shown earlier [5, 11, 15, 16], carnosine may specifically stimulate a natural defense system and increase the viability of cells under unfavorable conditions, features not belonging to histidine or β-alanine [5]. Carnosine treatment may be an effective way to slow senescence and to increase life span. The presence of carnosine in human tissues suggests that carnosine may be involved in natural anti-senescence mechanisms in human beings.

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