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Full Communications

Marine Collagen Peptides Prepared from Chum Salmon (*Oncorhynchus keta*) Skin Extend the Life Span and Inhibit Spontaneous Tumor Incidence in Sprague-Dawley Rats

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ABSTRACT To observe the effects of marine collagen peptides (MCPs) prepared from chum salmon (*Oncorhynchus keta*) skin on life span and spontaneous tumor incidence, Sprague-Dawley rats were fed diets supplemented with MCP at concentrations of 0%, 2.25%, 4.5%, and 9% (wt/wt) from the age of 4 weeks until natural death. There were 40 rats in each group (male:female ratio = 1:1). The results showed that the MCP did not significantly influence body weight or food consumption of rats of either sex throughout the life span; it did dose-dependently inhibit the age-related decrease in the activities of antioxidant enzymes and the age-related increase in the levels of lipid peroxidation product in both sexes. MCP notably increased the mean life span, the life span of the last 30% of the survivors, and the maximal life span; it decreased overall spontaneous tumor incidence of both sexes with significance in the 4.5% and 9% MCP-treated male groups and 9% MCP-treated female group. Compared to the control group, the incidence of death from tumors was decreased in MCP groups in comparison with the control group of both sexes. Therefore, we concluded that MCPs dose-dependently increase life span and decrease spontaneous tumor incidence in Sprague-Dawley rats. Moreover, the antioxidative property of MCPs may be responsible for the increased life span and protection against tumor development.

KEY WORDS: • ***antioxidant ability*** • ***longevity*** • ***marine collagen peptides*** • ***spontaneous tumor*** • ***Sprague-Dawley rat***

INTRODUCTION

THE SEARCH FOR EFFECTIVE and safe means of delaying or preventing the development of aging-related diseases, retarding aging processes, and prolonging life span has high priority in modern gerontology.[1] In recent years, the anti-aging effects of bioactive peptides have aroused much scientific interest and attention. As specific protein fragments, the endogenous peptide preparations from the pineal gland and thymus gland have been shown to produce antioxidant,[2] anticarcinogenic,[3] and immunomodulating activities,[4] which contribute to the capacity to increase life span in rats, mice, fruit flies, and the elderly with accelerated aging.[3,5–7]

In the past several decades, researchers have found that bioactive peptides derived from dietary animal or plant proteins can act as regulatory compounds with a range of hormone-like activities.[8,9] With marine species comprising nearly one-half of the total global biodiversity, the sea offers enormous resources to be explored for bioactive marine peptides from various marine organisms, fish, and fish waste products.[10,11] Marine collagen peptides (MCPs), rich in glycine, glutamic acid, proline, and hydroxylproline, are the compounds of oligopeptides enzymatically hydrolyzed from the collagen in scale, cartilage, and bone of marine fish.[12] In the present study, MCPs are preparations from enzymatically hydrolyzed skin of chum salmon (*Oncorhynchus keta*), a deep sea fish widely distributed in the north Pacific and Atlantic.[13] Various bioactive properties of MCPs have been described in previous studies, including antihypertensive,[14,15] anti-ulcer,[16] anti-skin aging,[17] and maintenance of normal bone integrity[18] effects. In addition, the fish collagen peptides were found to have high antioxidant activities in scavenging free radicals and inhibiting lipid peroxidation in *in vitro* studies.[19–21] Hence, the health-promoting potential and the antioxidative property of the fish collagen bioactive peptides suggest they may have positive effects on the life span of animals; however, at present there are no available data on the effects of food-derived bioactive peptides on the life span of animals.

When rats are treated with MCPs throughout their lifetime to investigate putative life extension effects, age-related spontaneous tumor incidence and development of adverse side effects should also be considered.[22] Although previous studies did not suggest carcinogenic potential of MCPs, the potential carcinogenicity of chronic treatments still should be assessed.[23] Therefore, the effects of MCPs on

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758the life span, spontaneous tumor development, and age-related oxidative status in Sprague-Dawley (S-D) rats were investigated in this study.

MATERIALS AND METHODS

Preparation procedure of test substance

MCPs provided by CF Haishi Biotechnology Ltd. Co. (Beijing, China) were derived from the skin of wild-caught chum salmon (*O. keta*) from the East China Sea (average body weight, 1.47 kg). In brief, the fish skin was cleaned, scaled, and cut into small pieces. The substance was then defatted according to the method of Klompong *et al*.[24] After being homogenized and emulsified in distilled water, the material was hydrolyzed by complex protease (3,000 U/g of protein), which included 7% trypsin, 65% papain, and 28% alkaline proteinase. The resultant hydrolysate was centrifuged to remove impurities. Subsequently, the liquid was separated through a ceramic membrane (pore size, 200 μm) for purification. The mineral salt was removed from the liquid through a procedure of nanofiltration. Then the purified liquid was condensed by cryoconcentration under vacuum at 70°C. After the condensed liquid was decolorized and deodorized with activated carbon and filtered, most of the water was removed by spray-drying, and the MCP powder used in the following investigations was obtained.

Characterization of MCPs

The MCP sample contains about 90% hydrolyzed protein, 6.0% ash, 1.4% carbohydrate, 2.5% water, and 0.1% fat. The peptide sample was analyzed by high-performance liquid chromatography (Waters Corp., Milford, MA, USA) using a Phenomenex (Torrance, CA, USA) C18 column (10×250 mm) with acetonitrile–0.05 mol/L phosphate buffer (pH 3.2) (10:90 vol/vol) as the mobile phase at a flow rate of 2.0 mL/minute and monitored at 208 nm using a tunable ultraviolet detector (model 486, Waters Corp.). Then the molecular weight distribution of the sample was ascertained by matrix-assisted laser desorption ionization time-of-flight mass spectrometry (model LDI-1700, Liner Scientific Inc., Reno, NV, USA). In addition, the amino acid composition was further analyzed by an automatic amino acid analyzer (model H835-50, Hitachi, Tokyo, Japan). The analysis of MCPs indicated that 86.5% of the molecular weights were distributed between 130 and 1,000 Da (Table 1). Amino acid composition analysis (Table 2) showed that MCPs was rich in Gly > Glu > Pro > Hyp > Asp > Ala > Arg.

Experimental animals and housing conditions

A total of 160 male and female S-D rats (4 weeks old), weighing 80–90 g, were obtained from the Animal Service of Health Science Center, Peking University, Beijing. Rats were housed two per plastic cages with free access to chow and tap water in a filter-protected air-conditioned room with controlled temperature (25 ± 2°C), relative air humidity (60 ± 5%), and 12-hour light/dark cycles (light on 07:30–19:30 hours). All animals were handled in accordance with the guidelines of the National Institutes of Health[25] and the guidelines of the Peking University Animal Research Committee (www.lab.pku.edu.cn).

Table 1. Molecular Distribution of Marine Collagen Peptides from Chum Salmon Skin

Molecular distribution	*Percentage (%)*
>1,000	8.95
800–1,000	19.50
600–800	31.05
400–600	17.90
130–400	18.01
<130	4.59

Experimental design

After a 1-week acclimation period, rats were randomly assigned to one of four groups (20 animals per sex per group): vehicle control group and three experimental groups. Control rats were fed with modified AIN93M rodent diet (Vital River Ltd. Co., Beijing) with casein as the main protein source. Rats in the three experimental groups were fed with either 2.25%, 4.5%, or 9% (wt/wt) MCPs in the AIN93M diet; casein was decreased by 2.25%, 4.5%, and 9% (wt/wt), respectively, compared with the control diet to maintain a constant dietary protein. The composition and the protein content of the experimental diets are presented in Table 3.

All animals were observed three times daily, at 08:00, 14:00, and 20:00 hours, for mortality and moribundity. Observations included changes in skin, fur, eyes, mucous membranes, somatomotor activity, and behavior patterns.

Table 2. Amino Acid Composition of Marine Collagen Peptides from Chum Salmon Skin

Amino acid	*Number of residues/100 residues*
Glycine	23.77
Glutamic acid	12.22
Proline	9.79
Hydroxyproline	7.51
Aspartic acid	7.29
Alanine	6.59
Arginine	6.08
Lysine	5.66
Leucine	4.64
Serine	4.23
Valine	2.94
Isoleucine	2.57
Threonine	2.53
Phenylalanine	2.51
Histidine	1.61
Methionine	0.03
Tyrosine	0.03

Table 3. Ingredients of Diets Administered During the Experimental Period

	Control		*2.25% MCPs*		*4.5% MCPs*		*9% MCPs*	
Ingredient	*Ingredient (%)*	*Protein (%)*	*Ingredient (%)*	*Protein (%)*	*Ingredient (%)*	*Protein (%)*	*Ingredient (%)*	*Protein (%)*
Cornstarch (0.74% protein)	35.6	0.26	35.6	0.26	35.6	0.26	35.6	0.26
Casein lactic (90% protein)	22.22	20.00	19.97	17.97	17.72	15.95	13.22	11.90
MCPs (90% protein)	0	0	2.25	2.03	4.5	4.05	9	8.10
Dextrin	15.19		15.19		15.19		15.19	
Granular sugar	10		10		10		10	
Fiber	5		5		5		5	
Soy oil	4.24		4.24		4.24		4.24	
L-Cystine	3		3		3		3	
Choline bitartrate	0.25		0.25		0.25		0.25	
AIN-93 Vitamin mix	1		1		1		1	
AIN-93 Mineral mix	3.5		3.5		3.5		3.5	
Total protein (%)	100	20.26	100	20.26	100	20.26	100	20.26

MCPs, marine collagen peptides.

759Body weight and food consumption were recorded weekly in the first 6 months and every 2 weeks thereafter.

All animals were examined weekly for the presence of palpable tumors (including mammary and skin/subcutaneous tumors). Special attention was paid to the palpable mass development during the study. The onset time, location, size, texture, and progression of each palpable mass were recorded throughout the study period. The onset time was defined as the date (or survival time) when the palpable mass could be palpated by three checkers and the three dimensions were larger than 5 mm. The growth time was defined as the period from the onset of a palpable mass until death from any cause.

All animals were kept under observation until natural death or until they showed predefined symptoms indicative of non-recoverable health problems or prolonged pain (*i.e.*, ''end points'' such as rapid excessive weight loss, dehydration, inability to ambulate and obtain food and water, labored respiration, infected or necrotic tumors, and tumors that impair ability to walk with normal gait).[26] The date of each death was recorded, and the mean overall life span, the mean life span of the last 30% of the animals, and the maximal life span were evaluated.

Serum oxidative status evaluations

Blood samples for serum oxidative stress evaluation were collected from the lateral tail vein every 6 months during the study. The animals were fasted overnight prior to the collection of blood samples. Serum oxidative parameters, including glutathione peroxidase (GSH-Px), superoxide dismutase (SOD), and thiobarbituric acid-reactive substances (TBARS), were examined by the commercial reagent kits (Jiancheng Institute of Biotechnology, Nanjing, China). The principles of these kits were as follows.

The activity of GSH-Px is reflected by the speed of the enzymatic reaction in which GSH-Px promotes reduced glutathione to generate oxidized glutathione. The remnant reduced glutathione can react with 5,5′-dithiobis2-nitrobenzoic acid) to form the stable product with the maximal absorption at 412 nm.[27]

The method for measuring SOD activity uses xanthine and xanthine oxidase to generate superoxide radicals that react with 2-(4-iodophenyl)-3-(4-nitrophenol)-5-phenyltetrazolium chloride. This reaction gives a red formazan dye product with the maximal absorbance at 550 nm. The SOD activity is then measured through the degree of inhibition of the reaction.[28]

The level of TBARS is expressed as malondialdehyde (MDA) equivalents, which are the products of lipid peroxidation as well as other breakdown products from oxidatively modified proteins, carbohydrates, and nucleic acids. MDA can react with thiobarbituric acid to form TBARS with the maximal absorption at 532 nm.[29]

Pathomorphological examination

A full necropsy was done on all animals that died spontaneously or were sacrificed in moribund status. At necropsy, the skin and all the internal organs and cavities were examined. The three-dimensional sizes of all tumors were measured with a caliper. The geometric volume of tumor was determined using the formula: length $\times$ width $\times$ height $\times$ 0.5326, assuming a hemi-ellipsoidal shape.[30] All tumors, as well as the tissues and organs with suspected tumors, were excised and fixed in 10% buffered formalin. Then the tissues were embedded in paraffin, sectioned at 5 μm with a rotary microtome, and stained with hematoxylin and eosin for microscopic examination (Olympus BH2 microscope, Olympus Optical Co., Ltd., Tokyo). According to the recommendations of the International Agency of Research on Cancer, tumors were designated as ''fatal'' (those that were directly or indirectly responsible for the animal's death) or ''incidental'' (those that did not cause death and death had arisen from an unrelated cause).[31,32] The primary causes of spontaneous death were determined by the symptoms in moribund status as well as the gross and histopathologic findings.

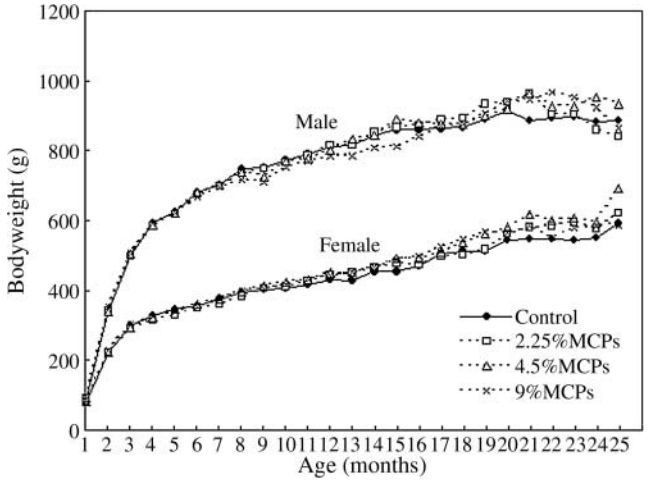

FIG. 1. Growth curves for Sprague-Dawley rats treated with MCPs for 24 months: control (•), 2.25% MCP-treated (■), 4.5% MCPs (△), and 9% MCPs (×). Twenty rats were used in each group of each sex. The values for body weight represent mean values, and the data were analyzed for signficance of differences by one-way analysis of variance test. Multiple comparison by Dunnett's *t* test was used to evaluate the difference between MCP-treated groups and the control group. All $P > .05$ compared with control values.

760Statistical analysis

Statistical analyses were performed using SPSS software (version 13.0, SPSS Inc., Chicago, IL, USA). Variances in the measurement data (*i.e.*, body weight, food consumption, serum oxidative stress parameters, and the size, onset time, and growth time of tumor) were checked for homogeneity by Bartlett's test. When the data were homogeneous, the one-way analysis of variance test and multiple comparison of Dunnett's *t* test were used. The heterogeneous cases were analyzed with the Kruskal-Wallis rank sum test. Categorical data, like the frequency of tumor and frequency of death causes, were compared using Fisher's exact probability test. For longevity analysis, Kaplan-Meier survival analysis was used with log-rank tests for group differences. All reported *P* values were two-sided. A value of $P < .05$ was considered significant.

RESULTS

Effects of MCPs on age-related body weight dynamics

There was no remarkable difference in general condition and behavior between the control and MCP-treated rats of both sexes. As shown in the growth curves for S-D rats (Fig. 1), there were no significant differences in body weight among groups of either sex throughout the experimental period ($P > .05$).

Effects of MCPs on age-related food consumption dynamics

There was no MCP dose-related change in food consumption in either sex throughout the experiment. During the entire period of observation, no significant difference in food consumption was indicated from the comparison between the vehicle-treated control group and the MCP-treated groups ($P > .05$). Only at the age of 3 months was the food consumption of 2.25% and 9% MCP-treated female groups significantly lower than the control group ($P < .05$), while no dose-related change was indicated (Table 4).

Effects of MCPs on age-related oxidative status in serum

Investigations of the serum activities of SOD and GSH-PX and the serum level of TBARS were performed at the age of 6, 12, 18, and 24 months.

Serum GSH-PX activity (Table 5) exhibited a significant age-related decrease among all groups of both sexes. At the age of 6 months, the activity of GSH-PX did not differ between the control and MCP-treated groups of either sex. At the ages of 12, 18, and 24 months, the GSH-PX enzyme activity of MCP-treated rats was significantly higher compared with the control group of males (12 months, 4.5% and 9% MCP-treated vs. control; 18 months, 4.5% and 9% MCP-treated vs. control; 24 months, 4.5% and 9% MCP-treated vs. control) and females (12 months, 2.25%, 4.5%, and 9% MCP-treated vs. control; 18 months, 4.5% and 9% MCP-treated vs. control; 24 months, 2.25%, 4.5%, and 9% MCP-treated vs. control).

Treatment with MCPs also resulted in an elevation in the activity of SOD enzyme that decreased with age in all

TABLE 4. FOOD CONSUMPTION DYNAMICS IN SPRAGUE-DAWLEY RATS TREATED WITH DIFFERENT LEVELS OF MARINE COLLAGEN PEPTIDES

	Food consumption (g/kg of body weight/day)							
Sex, MCPs (%)	*3 months*	*6 months*	*9 months*	*12 months*	*15 months*	*18 months*	*21 months*	*24 months*
Female								
0	75.29 ± 8.63	59.04 ± 5.67	56.78 ± 6.40	50.09 ± 8.94	46.98 ± 6.05	43.01 ± 4.23	44.58 ± 7.20	42.23 ± 9.20
2.25	69.58 ± 8.33*	57.99 ± 7.02	60.05 ± 9.41	50.67 ± 9.43	45.16 ± 7.50	43.08 ± 8.30	38.77 ± 9.73	37.49 ± 10.27
4.5	74.02 ± 10.22	59.53 ± 8.97	57.96 ± 12.00	53.00 ± 10.60	47.39 ± 11.12	44.16 ± 9.25	40.17 ± 9.64	40.22 ± 9.47
9	66.60 ± 7.52*	55.85 ± 6.11	57.21 ± 8.25	49.98 ± 7.90	43.42 ± 7.56	44.39 ± 10.68	38.87 ± 8.16	34.49 ± 6.81
Male								
0	55.59 ± 5.28	47.92 ± 5.01	46.97 ± 6.30	41.82 ± 8.50	39.42 ± 9.88	40.60 ± 7.29	36.96 ± 7.45	35.81 ± 5.20
2.25	55.27 ± 5.55	44.56 ± 4.20	47.30 ± 5.13	41.90 ± 4.43	39.30 ± 4.83	35.88 ± 6.17	35.05 ± 5.51	38.08 ± 5.69
4.5	55.01 ± 5.75	44.39 ± 5.68	47.10 ± 6.93	42.53 ± 7.40	34.93 ± 5.76	37.03 ± 5.37	35.33 ± 4.99	27.95 ± 6.15
9	55.59 ± 4.04	45.30 ± 4.34	47.63 ± 5.62	41.81 ± 6.53	38.61 ± 4.64	42.94 ± 5.55	34.06 ± 3.80	32.08 ± 6.53

Data are mean ± SD values. Data were analyzed by one-way analysis of variance test. Multiple comparison by Dunnett's *t* test was used to evaluate the difference between MCP-treated groups and the control group.

Significant difference compared with the control group: $*P < .05$.

Table 5. Serum Glutathione Peroxidase Activity in Sprague-Dawley Rats Treated with Different Levels of Marine Collagen Peptides

	GSH-PX activity (U/mL)							
	6 months		*12 months*		*18 months*		*24 months*	
Sex, MCPs (%)	n	*Value*	n	*Value*	n	*Value*	n	*Value*
Male								
0	20	1,940.85 ± 211.96	20	1,353.00 ± 109.82	18	997.75 ± 179.05	9	886.29 ± 217.66
2.25	20	1,956.20 ± 184.53	20	1,460.74 ± 222.41	19	1,151.68 ± 187.03	13	1,074.32 ± 141.82
4.5	20	1,902.83 ± 178.30	20	1,695.41 ± 162.50*	19	1,401.56 ± 222.26*	13	1,299.32 ± 201.60**
9	20	1,923.81 ± 183.74	20	1,700.27 ± 200.45*	18	1,571.69 ± 241.37***	14	1,344.78 ± 188.09***
Female								
0	20	1,934.58 ± 171.77	20	1,323.14 ± 166.01	16	1,183.03 ± 167.57	11	936.25 ± 138.84
2.25	20	1,969.34 ± 187.23	20	1,552.03 ± 225.13*	19	1,289.05 ± 165.12	14	1,162.60 ± 230.50*
4.5	20	2,115.42 ± 135.39	20	1,622.66 ± 169.03**	18	1,444.71 ± 183.98**	11	1,243.66 ± 173.57**
9	20	1,955.20 ± 213.82	20	1,568.66 ± 101.11**	19	1,659.81 ± 207.43***	12	1,261.96 ± 113.40**

Data are mean ± SD values. Data were analyzed by one-way analysis of variance test.
Significant difference compared with the control group: *$P < .05$, **$P < .01$, ***$P < .001$.
GSH-PX, glutathione peroxidase.

761groups (Table 6). No remarkable difference in SOD enzyme activity was observed at the age of 6 months between the control and MCP-treated groups of either sex. At the age of 18 and 24 months, the serum SOD activity of MCP-treated rats remained at significantly higher levels versus the control group of males (18 months, 2.25%, 4.5%, and 9% MCP-treated vs. control; 24 months, 4.5% and 9% MCP-treated vs. control) and females (18 months, 4.5% and 9% MCP-treated vs. control; 24 months, 4.5% and 9% MCP-treated vs. control).

In contrast, there was a significant age-related increase in the TBARS level of control rats (Table 7). However, this increasing tendency in the TBARS level was attenuated in MCP-treated rats. When compared with controls at the ages of 12, 18, and 24 months, serum TBARS levels were significantly lower in MCP-treated groups of males (12 months, 4.5% and 9% MCP-treated vs. control; 18 months, 2.25%, 4.5%, and 9% MCP-treated vs. control; 24 months, 2.25%, 4.5%, and 9% MCP-treated vs. control) and females (12 months, 4.5% and 9% MCP-treated vs. control; 18 months, 2.25%, 4.5%, and 9% MCP-treated vs. control; 24 months, 2.25%, 4.5%, and 9% MCP-treated vs. control).

Survival and longevity of S-D rats

The survival analysis of rats from the control and MCP-treated groups was done for (1) all animals in each group and (2) tumor-bearing animals or tumor-free animals.

Survival of all animals. The results in Table 8 showed that the mean life spans of rats treated with MCPs were obviously increased compared with controls. The effect of MCPs on longevity was significant with genders pooled

Table 6. Serum Superoxide Dismutase Activity in Sprague-Dawley Rats Treated with Different Levels of Marine Collagen Peptides

	SOD activity (U/mL)							
	6 months		*12 months*		*18 months*		*24 months*	
Sex, MCPs (%)	n	*Value*	n	*Value*	n	*Value*	n	*Value*
Male								
0	20	237.76 ± 17.27	20	172.95 ± 11.71	18	132.86 ± 18.37	9	141.76 ± 12.83
2.25	20	236.05 ± 15.90	20	177.94 ± 10.33	19	156.75 ± 21.66*	13	155.54 ± 10.44
4.5	20	241.34 ± 18.47	20	187.70 ± 12.69	19	172.86 ± 19.26**	13	159.37 ± 11.39*
9	20	250.25 ± 13.03	20	183.89 ± 15.31	18	163.81 ± 22.78**	14	160.89 ± 16.10*
Female								
0	20	244.08 ± 21.83	20	177.70 ± 13.27	16	140.74 ± 11.20	11	135.68 ± 20.40
2.25	20	255.41 ± 15.05	20	184.36 ± 12.39	19	160.75 ± 22.58	14	153.63 ± 12.54
4.5	20	253.71 ± 13.14	20	192.03 ± 13.21	18	171.61 ± 18.85**	11	169.27 ± 10.44**
9	20	259.26 ± 15.56	20	186.60 ± 15.60	19	169.53 ± 22.63**	12	166.74 ± 17.38**

Data are mean ± SD values. Data were analyzed by one-way analysis of variance test.
Significant difference compared with the control group: *$P < .05$, **$P < .01$.
SOD, superoxide dismutase.

Table 7. Serum Malondialdehyde Level In Sprague-Dawley Rats Treated with Different Levels of Marine Collagen Peptides

	MDA (nmol/mL)							
	6 months		12 months		18 months		24 months	
Sex, MCPs (%)	n	Value	n	Value	n	Value	n	Value
Male								
0	20	15.35 ± 0.39	20	20.59 ± 1.92	18	28.97 ± 1.12	9	28.25 ± 1.04
2.25	20	15.28 ± 0.36	20	19.05 ± 2.03	19	25.84 ± 1.27**	13	26.09 ± 1.32**
4.5	20	15.37 ± 0.39	20	17.25 ± 1.95**	19	27.36 ± 1.69*	13	25.98 ± 1.10**
9	20	14.98 ± 0.41	20	18.65 ± 1.70*	18	25.73 ± 1.96**	14	26.16 ± 1.55*
Female								
0	20	14.95 ± 0.17	20	20.44 ± 1.96	16	27.76 ± 2.40	11	29.25 ± 1.50
2.25	20	15.21 ± 0.25	20	19.11 ± 1.10	19	24.67 ± 1.70**	14	26.68 ± 2.22*
4.5	20	15.08 ± 0.25	20	17.38 ± 2.14**	18	25.79 ± 1.55*	11	25.84 ± 1.06**
9	20	14.93 ± 0.37	20	18.42 ± 1.83*	19	25.83 ± 1.49*	12	27.09 ± 1.29*

Data are mean ± SD values. Data were analyzed by one-way analysis of variance test.
Significant difference compared with the control group: *$P < .05$, **$P < .01$.
MDA, malondialdehyde.

762(log-rank test, $\chi^2 = 9.146$, $P = .027$; 2.25% MCP-treated vs. control, $P = .016$; 4.5% MCP-treated vs. control, $P = .051$; 9% MCP-treated vs. control, $P = .008$). When gender difference was considered, the significance was attenuated because of the smaller sample size.

Moreover, the survival curves in Figure 2 indicated that MCPs exerted a more prominent effect on the long-lived subpopulation compared to the short-lived subpopulation. In accordance with the result, survival dynamics were similar in all groups up to the age of 28 months. After this age, the number of survivors in MCP-treated groups was considerably higher compared to control group of both sexes, with statistical significance in males (Fisher's exact test, $\chi^2 = 9.366$, $P = .027$; 9% MCP-treated vs. control). Compared with the control male group, the average life span in the last 30% of the male survivors (Table 8) slightly increased by 2.30 months (69 days, +8.0%), 2.34 months (70.3 days, +8.2%), and 2.19 months (65.8 days, +7.6%) with treatment with 2.25%, 4.5%, and 9% MCPs, respectively. A more notable effect of MCPs on the long-lived survivors was revealed from the average life span of the last 30% of the female rats, with prolongation of 4.1 months (123.2 days, +14.4%), 3.56 months (106.7 days, +12.4%), and 4.2 months (126.4 days, +14.7%) in the 2.25%, 4.5%, and 9% MCP-treated groups, respectively. When the gender difference was disregarded, a significant longevity extension effect of MCPs on the last 30% of the surviors was observed in all treated groups.

The last rat in the control male group died at the age of 967 days (32.2 months), whereas the MCP-treated males at

Table 8. Parameters of Life Span in Sprague-Dawley Rats Treated with Different Levels of Marine Collagen Peptides

Sex, MCPs (%)	n	Mean ± SE life span (days)	Median (days)	Mean ± SE life span of last 30% of survivors (days)	Maximum life span (days)
Male					
0	20	709.9 ± 30.9	703	861.7 ± 31.9	967
2.25	20	773.4 ± 31.5	799	930.7 ± 25.7	1,011
4.5	20	771.1 ± 30.4	770	932.0 ± 25.6	1,029
9	20	795.2 ± 32.1	840	927.5 ± 20.9	1,000
Female					
0	20	711.8 ± 32.2	731	857.3 ± 26.1	954
2.25	20	783.9 ± 37.7	782	980.5 ± 29.9*	1,087
4.5	20	766.9 ± 37.8	780	964.0 ± 41.5*	1,079
9	20	783.1 ± 37.9	738	983.7 ± 33.9*	1,092
Male + female					
0	40	710.8 ± 22.0	707	859.5 ± 19.7	967
2.25	40	778.7 ± 24.3*	795	955.6 ± 20.3**	1,087
4.5	40	769.0 ± 24.0	772	948.0 ± 23.7*	1,079
9	40	789.2 ± 24.5**	806	955.6 ± 20.8*	1,092

Data are mean ± SE values. Data were analyzed by Kaplan-Meier survival analysis, and the log-rank test was used to evaluate the difference between MCP-treated groups and the control group.
Significant difference compared with the control group: *$P < .05$, **$P < .01$.

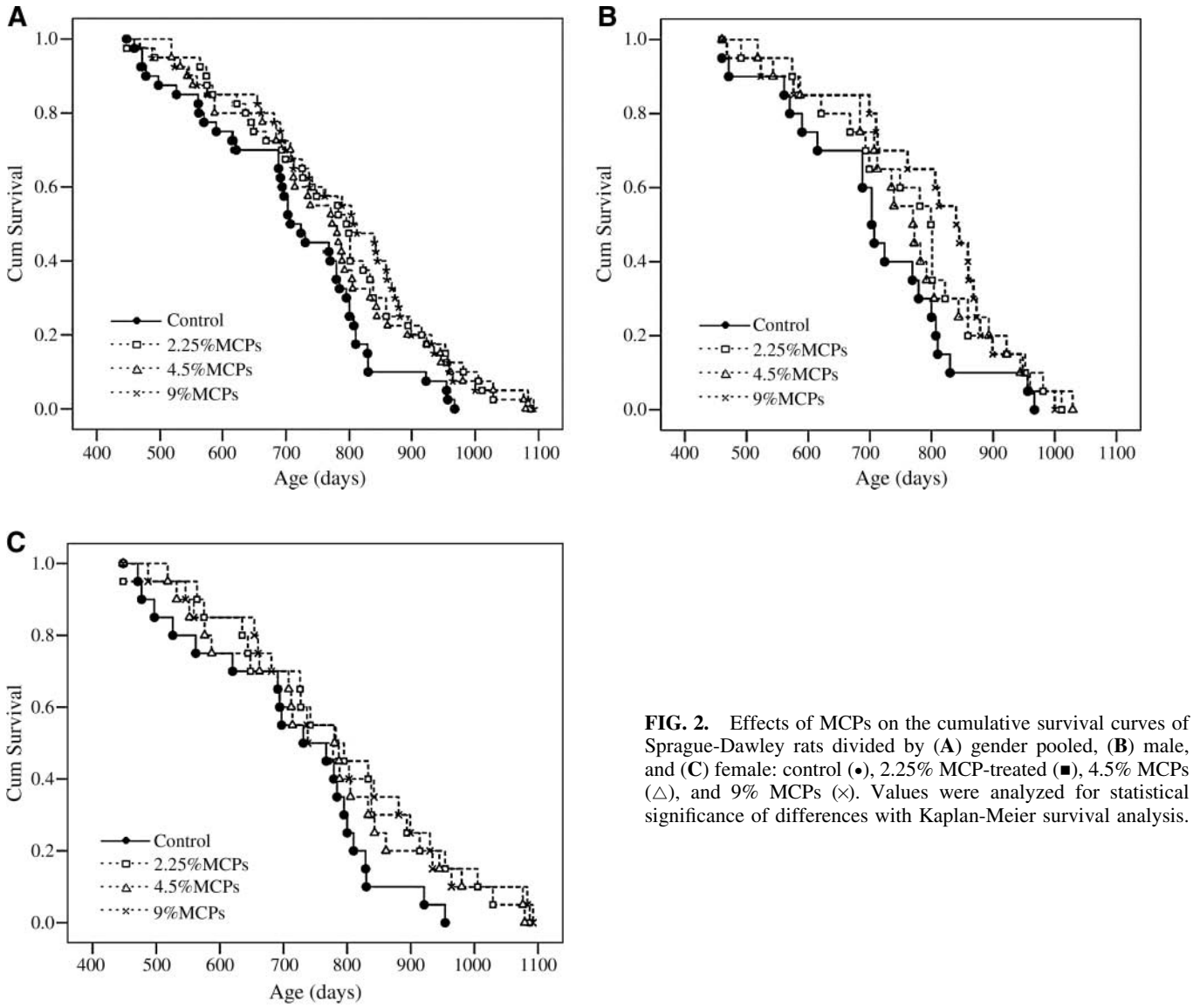

FIG. 2. Effects of MCPs on the cumulative survival curves of Sprague-Dawley rats divided by (**A**) gender pooled, (**B**) male, and (**C**) female: control (•), 2.25% MCP-treated (■), 4.5% MCPs (△), and 9% MCPs (×). Values were analyzed for statistical significance of differences with Kaplan-Meier survival analysis.

763this age survived 10%, 5%, and 5% longer. Under the effect of MCPs, the maximal life span was extended by 44, 62, and 33 days (1.47, 2.07, and 1.1 months, respectively) in the 2.25%, 4.5%, and 9% MCP-treated male rats, respectively. As for the control females, the rat with the maximal life span survived 954 days (31.8 months), whereas 15% of the MCP-treated female rats survived to this age. Specifically, the last rats in the 2.25%, 4.5%, and 9% MCP-treated female groups died at the ages of 1,087, 1,079, and 1,092 days, with increases of 133, 125, and 138 days (4.43, 4.17, and 4.6 months, respectively) compared with the maximal life span of female controls.

Survival of tumor-bearing and tumor-free animals. The overall mean life spans of tumor-bearing animals were 813.1 ± 28.9 days for males and 792.4 ± 23.4 days for females. It should be noted that the tumor-free animals had a much shorter life span (728.6 ± 16.2 days for males and 712.5 ± 28.1 days for females) compared with the tumor-bearing animals (log-rank test, $P < .05$). As shown in the survival curve of tumor-bearing animals, MCPs significantly extended the life spans of tumor-bearing animals of both sexes (Fig. 3). The mean life spans of tumor-bearing rats treated with MCPs were dose-dependently prolonged compared with the controls, with significance in the 9% MCP-treated group of both sexes (log-rank test, $P < .05$). As for the tumor-free rats, the significant longevity extension effect was observed in the 9% MCP-treated male group as well as the 2.25% and 4.5% MCP-treated female groups (log-rank test, $P < .05$) (Tables 9 and 10).

Spontaneous tumor development in S-D rats

The presence of neoplastic lesions at anatomic sites was revealed by macroscopic and histological analyses (Tables 9 and 10). Treatment with MCPs exerted a certain inhibitory

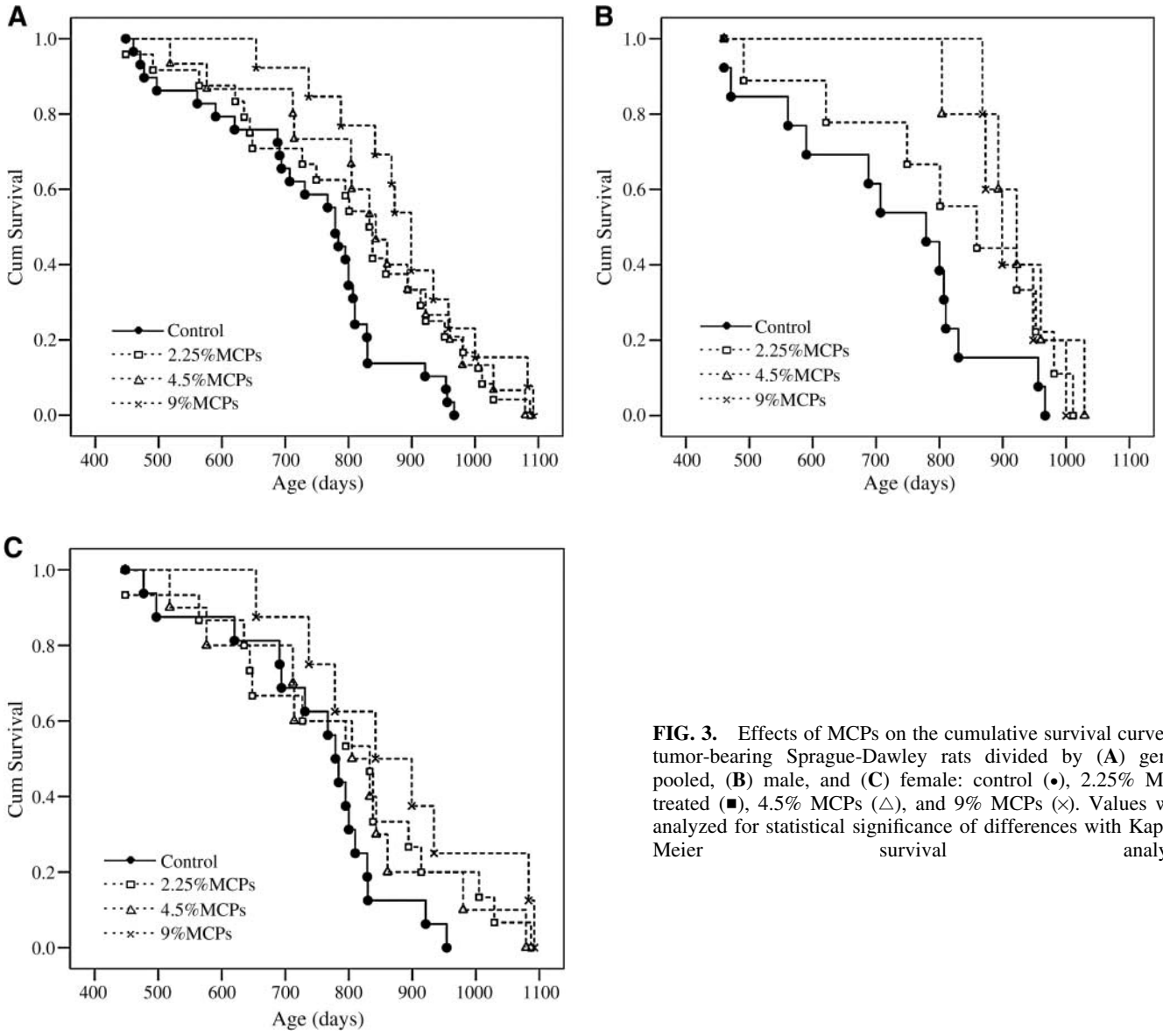

FIG. 3. Effects of MCPs on the cumulative survival curves of tumor-bearing Sprague-Dawley rats divided by (**A**) gender pooled, (**B**) male, and (**C**) female: control (•), 2.25% MCP-treated (■), 4.5% MCPs (△), and 9% MCPs (×). Values were analyzed for statistical significance of differences with Kaplan-Meier survival analysis.

764effect on spontaneous carcinogenesis in rats, which was manifested by a decreased incidence of total tumors in both sexes. Compared with the control group, significantly reduced overall tumor incidences were observed in the 4.5% and 9% MCP-treated male groups (Fisher's exact test, $\chi^2 = 8.874$, $P = .031$; 4.5% and 9% MCP-treated vs. control, $P < .05$) as well as the 9% MCP-treated female group (Fisher's exact test, $\chi^2 = 9.220$, $P = .023$; 9% MCP-treated vs. control, $P < .05$).

The results showed that MCPs did not increase the frequency of malignant or benign tumors. Moreover, the occurrence of benign and malignant tumors in all MCP-treated groups of both sexes tended to decrease compared with the controls. Disregarding the division by sex, treatment with 2.25%, 4.5%, and 9% MCPs resulted in 2-, 2.7-, and eightfold decreases in the malignant tumor incidence compared with the vehicle treatment (Fisher's exact tests, $\chi^2 = 6.605$, $P = .088$).

Mammary tumors, the most frequent neoplastic lesions in female S-D rats, were present in nine (45%) control females. Animals with mammary tumors accounted for 40%, 35%, and 20.0% of the 2.25%, 4.5%, and 9% MCP-treated rats, respectively, which indicated a decreased tendency of mammary tumor incidence. No remarkable difference was found in the onset time of mammary tumor between the MCP- and vehicle control-treated female rats. At the time of necropsy, the volume of the mammary tumor per rat in the 2.25% and 4.5% MCP-treated groups was significantly decreased compared with the control females ($P < .05$). In addition, the average growth time of the mammary tumor per rat in the MCP-treated groups tended to be longer than that of control rats. Consistent with the mammary tumor results, the skin/subcutaneous tumors in MCP-treated rats at necropsy exhibited a decreased tumor size and longer growth time compared to the controls, although the limited sample size lowered the statistical sensitivity.

Table 9. Spontaneous Neoplastic Lesions in Sprague-Dawley Male Rats Treated with Different Levels of Marine Collagen Peptides

Site	Lesion	MCPs (%) 0 (n = 20)	2.25 (n = 20)	4.5 (n = 20)	9 (n = 20)
Skin/subcutis	Adenoma (B)	1	1	0	1
	Fibroma (B)	1	2	3 (4)	2
	Lipoma (B)	2	1	0	0
	Total tumor size/rat (cm^3)[a]	69.30 ± 45.76	53.98 ± 34.39	37.19 ± 50.71	40.74 ± 64.25
	Onset time (day)[a]	580.0 ± 62.0	627.3 ± 98.3	690.7 ± 77.1	639.3 ± 56.6
	Growth time (day)[a]	232.5 ± 46.5	274.0 ± 62.4	258.0 ± 75.0	260.3 ± 56.1
Pituitary gland	Adenoma (B)	4	3	3	2
	Tumor size/rat (cm^3)[a]	0.38 ± 0.16	0.30 ± 0.17	0.27 ± 0.18	0.14 ± 0.11
Liver	Adenoma (B)	2	0	1	1
	Tumor size/rat (cm^3)[a]	1.38 ± 0.05	—	0.36	0.62
	Adenocarcinoma (M)	1	0	0	0
	Tumor size/rat (cm^3)	23.05	—	—	—
Lung	Lung metastases (M)	0	1	0	0
	Tumor size/rat (cm^3)	—	0.15	—	—
Pancreas	Adenocarcinoma (M)	3 (4)	2	1	0
	Tumor size/rat (cm^3)[a]	14.16 ± 9.86	11.82 ± 7.68	12.78	—
Adrenal gland	Cortical adenoma (B)	1	1	0	2
	Tumor size/rat (cm^3)[a]	0.18	0.10	—	0.23 ± 0.05
	Pheochromocytomas (M)	0	1	0	0
	Tumor size/rat (cm^3)	—	0.09	—	—
Prostate	Adenoma (B)	1	0	1	0
	Tumor size/rat (cm^3)	3.74	—	0.83	—
Bladder	Cell carcinoma (M)	0	1	0	0
	Tumor size/rat (cm^3)	—	0.04	—	—
Abdominal cavity	Vascular tumor (M)	2	1	0	0
	Tumor size/rat (cm^3)[a]	357.91 ± 78.70	102.26	—	—
Systemic	Leukemia (M)	1	0	0	0
Number of benign tumors		12	8	9	8
Number of rats with benign tumors		11	8	4	5
Number of malignant tumors		8	6	1	0
Number of rats with malignant tumors		4	2	1	0
Number of tumors		20	14	10	8
Number of tumor-bearing rats		13	9	$5^{\#}$	$5^{\#}$
Number of tumors per tumor-bearing rat		1.5	1.6	2	1.6
Mean life span of tumor-bearing rat (days)[b]		725.1 ± 45.8	820.8 ± 58.3	921.6 ± 37.2	$919.6 \pm 25.7^{\triangle}$
Mean life span of tumor-free rat (days)[b]		681.7 ± 25.5	734.6 ± 29.3	720.9 ± 28.7	$753.7 \pm 36.1^{\triangle}$

Data are the number of rats with the lesions where the number is parentheses is the total number of lesions in the group when at least one rat has more than one lesion, or [a]mean ± SD or [b]mean ± SE values.

Significant difference compared with the control group (by Fisher's exact probability test): $^{\#}P < .05$.

Significant difference compared with the control group (by Kaplan-Meier survival analysis, log-rank test): $^{\triangle}P < .05$.

B, benign tumor; M, malignant tumor.

765Pituitary tumor is another frequent neoplastic lesion in S-D rats of both sexes. Our results showed that the incidences of pituitary gland adenoma in the control group were 20% in males and 30% in females. Female rats treated with 2.25%, 4.5%, and 9% MCPs exhibited 1.5-, six-, and sixfold decreases in the incidence of pituitary gland adenomas compared with the female control rats, with significance for the 4.5% and 9% MCP-treated groups (Fisher's exact χ^2 tests, $P < .05$). At the time of necropsy, there was no marked difference in the volume of pituitary tumor between the MCP-treated groups and the control group of either sex. In addition, no other statistical significance was found in the incidence or size of other neoplasms.

Probable causes of spontaneous deaths

As shown in Table 11 and Table 12, the causes of spontaneous death were summarized into two categories: neoplastic and non-neoplastic lesions. It should be noted that some cases of spontaneous death with causes involved with a complex interaction of multiple pathologies were defined as undetermined causes. The percentage of undetermined death cause did not differ significantly among groups ($P > .05$). The cause of death analysis had been established in more than 80% of the cases.

In all groups, the main causes of death were neoplastic lesions, accounting for 50%, 30%, 15%, and 15% of deaths in the control and MCP-treated groups, respectively, among

Table 10. Spontaneous Neoplastic Lesions in Sprague-Dawley Female Rats Treated with Different Levels of Marine Collagen Peptides

		MCPs (%)			
Site	*Lesion*	*0* (n = *20*)	*2.25* (n = *20*)	*4.5* (n = *20*)	*9* (n = *20*)
Mammary gland	Adenoma (B)	5 (8)	4 (6)	3 (4)	2
	Fibroadenoma (B)	3 (6)	3 (7)	4 (6)	2
	Benign tumor size/ rat (cm^3)[a]	187.62 ± 55.97	87.21 ± 64.68*	116.55 ± 74.34*	137.15 ± 48.15
	Adenocarcinoma (M)	1	1	0	0
	Malignant tumor size/ rat (cm^3)	287.60	204.44	±	
	Total tumor size/rat (cm^3)[a]	198.73 ± 62.06	101.87 ± 72.83*	116.55 ± 74.34*	137.15 ± 48.15
	Onset time (day)[a]	534.1 ± 77.4	561.4 ± 80.5	542.9 ± 56.3	549.9 ± 98.8
	Growth time (day)[a]	267.7 ± 77.2	268.1 ± 80.0	272.4 ± 93.6	304.1 ± 84.1
Skin/Subcutis	Adenoma (B)	1	0	0	1
	Fibroma (B)	3	2	2	2
	Lipoma (B)	1	0	0	0
	Total tumor size/rat (cm^3)[a]	84.50 ± 39.45	69.44 ± 36.22	74.47 ± 87.24	47.04 ± 56.91
	Onset time (day)[a]	522.8 ± 83.5	644.0 ± 59.4	579.0 ± 52.3	643.7 ± 205.7
	Growth time (day)[a]	214.0 ± 54.1	232.0 ± 113.1	211.5 ± 12.0	237.0 ± 52.7
Pituitary gland	Adenoma (B)	6	4	1[#]	1[#]
	Tumor size/rat (cm^3)[a]	0.45 ± 0.15	0.42 ± 0.17	0.35	0.37
Liver	Adenoma (B)	3	0	0	1
	Tumor size/rat (cm^3)[a]	4.06 ± 2.70	—	—	1.07
Lung	Lung metastases (M)	0	1	0	0
	Tumor size/rat (cm^3)	—	0.15	—	—
Pancreas	Adenocarcinoma (M)	2	0	1	1
	Tumor size/rat (cm^3)[a]	11.91 ± 2.93	—	8.39	0.35
Adrenal gland	Cortical adenoma (B)	0	2	2	0
	Tumor size/rat (cm^3)[a]	—	0.04 ± 0.03	0.03 ± 0.01	—
	Pheochromocytomas (M)	1	1	0	0
	Cortical carcinoma (M)	1	1	1	0
	Tumor size/rat (cm^3)[a]	0.22 ± 0.05	0.35 ± 0.11	0.30	—
Ovarian	Luteinoma (B)	0	1	0	1
	Tumor size/rat (cm^3)	—	0.32	—	0.65
	Malignant teratoma (M)	1	0	0	0
	Tumor size/rat (cm^3)	0.47	—	—	—
Bladder	Cell carcinoma (M)	1	0	0	0
	Tumor size/rat (cm^3)	0.26	—	—	—
Abdominal cavity	Vascular tumor (M)	2	1	1	1
	Tumor size/rat (cm^3)[a]	197.13 ± 168.62	149.13	178.95	194.29
Systemic	Lymphoma (M)	1	0	0	0
Number of benign tumors		28	22	15	10
Number of rats with benign tumors		13	13	9	7
Number of malignant tumors		10	5	3	2
Number of rats with malignant tumors		4	2	2	1
Number of tumors		38	27	18	12
Number of tumor-bearing rats		16	15	10	8[#]
Number of tumors per tumor-bearing rat		2.4	1.8	1.8	1.5
Mean life span of tumor-bearing rats (days)[b]		748.7 ± 32.7	793.3 ± 46.9	800.8 ± 59.4	878.6 ± 55.2[△]
Mean life span of tumor-free rats (days)[b]		564.0 ± 48.1	755.8 ± 60.7[△]	741.7 ± 54.8[△]	719.5 ± 43.9

Data are the number of rats with the lesions where the number is parentheses is the total number of lesions in the group when at least one rat has more than one lesion, or [a]mean ± SD or [b]mean ± SE values.

Significant difference compared with the control group (by one-way analysis of variance): $*P < .05$.

Significant difference compared with the control group (by Fisher's exact probability test): $^{\#}P < .05$.

Significant difference compared with the control group (by Kaplan-Meier survival analysis, log-rank test): $^{\triangle}P < .05$.

B, benign tumor; M, malignant tumor.

766males and 65%, 45%, 35%, and 15%, respectively, among females. These tumors were benign in approximately 68% of the cases, which mainly consisted of pituitary and mammary/subcutaneous adenomas. Pituitary tumor was found to be the most common neoplastic cause of death in S-D rats for males (20%) and females (30%) in the control group. As described in previous studies, pituitary tumors are fatal in S-D rats when the mean diameter of the lesions exceeds 7 mm because the large intracranial mass produces cerebral compression.[33] Compared with the vehicle-treated control group, the frequency of death due to pituitary tumor in the MCP-treated groups decreased in a dose-dependent

Table 11. Primary Causes of Spontaneous Deaths of Male Sprague-Dawley Rats Treated with Different Levels of Marine Collagen Peptides

	MCPs (%)			
Death cause	*0 (n = 20)*	*2.25 (n = 20)*	*4.5 (n = 20)*	*9 (n = 20)*
Neoplastic lesion				
Benign				
Pituitary gland	4	3	2	2
Skin/subcutis	2	1	0	1
Subtotal	6	4	2	3
Malignant				
Liver	1			
Pancreas	1	1	1	
Abdominal metastases	1	1		
Leukemia	1			
Subtotal	4	2	1	
Total	10	6	3	3
Non-neoplastic lesion				
Chronic nephropathy	3	5	4	5
Emphysema	1	2	3	3
Hepatocirrhosis	1	1	2	2
Hepatic steatosis	2	1	2	2
Megalosplenia			1	
Intestinal obstruction		1	1	1
Total	7	10	13	13
Undetermined	3	4	4	4

Data are the numbers of deaths.

Table 12. Primary Causes of Spontaneous Deaths of Female Sprague-Dawley Rats Treated with Different Levels of Marine Collagen Peptides

	MCPs (%)			
Cause of death	*0 (n = 20)*	*2.25 (n = 20)*	*4.5 (n = 20)*	*9 (n = 20)*
Neoplastic lesion				
Benign				
Pituitary gland	6	4	1	1
Mammary gland	3	2	2	1
Skin/subcutis		1	1	
Subtotal	9	7	4	2
Malignant				
Mammary gland	1	1		
Pancreas	1		1	
Lymphoma	1			
Abdominal metastases	1	1	1	1
Subtotal	4	2	2	1
Total	13	9	6	3
Non-neoplastic lesion				
Chronic nephropathy	2	3	3	4
Emphysema	1	1	2	2
Hepatocirrhosis		2	1	2
Hepatic steatosis	2	2	3	3
Megalosplenia			1	1
Diabetes				1
Total	5	8	10	13
Undetermined	2	3	4	4

Data are the numbers of deaths.

767manner when genders were pooled (Fisher's exact test, $\chi^2 = 9.655$, $P = .087$).

For female rats, mammary gland tumor was the second most common cause of death. Additionally, a few cases of skin/subcutaneous tumors were lethal for both sexes. The mammary gland tumor as well as skin/subcutaneous tumor might result in a moribund condition of the individual when the large mass had impeded feeding and/or ulcerated and then underwent necrosis.[33] Compared with the control group, the frequencies of the large masses of mammary or skin/subcutaneous benign tumors as causes of death were decreased to some extent in MCP-treated rats.

As another type of cause of neoplastic death, malignant and systemic (leukemia and lymphoma) tumors were fatal by invasion of or metastasis to vital organs. Compared with the vehicle-treated groups, the incidences of death from malignant and systemic tumors in the MCP-treated groups of both sexes were obviously decreased (Fisher's exact test, $\chi^2 = 6.605$, $P = .088$).

Among the rats bearing nonfatal tumor or the tumor-free rats, the deaths were mainly attributed to vital organ failure caused by age-related non-neoplastic lesions, such as, for example, chronic nephropathy, hepatocirrhosis, and emphysema. Our results showed that a higher percentage of rats in the MCP-treated groups died of non-neoplastic lesions compared with controls.

DISCUSSION

With various bioactive properties, marine bioactive peptides from fish skin have gained increasing popularity as dietary supplements. Therefore, if life span extension and antitumor effects of the bioactive peptides were revealed, it would open a new avenue for developing value-added foods or health-promoting foods in the field of geriatric nutrition.

In the present study, the characterization of MCPs enzymatically hydrolyzed from chum salmon (*O. keta*) skin was identical with that of research by Shen *et al*.[12] The results of our study showed that long-term administration of MCPs increased the survival and prolonged the life span of the long-lived subpopulation. Moreover, MCPs were observed to inhibit the incidence and development of spontaneous tumors in S-D rats. To the best of our knowledge, this is the first murine study of the effects of MCPs on life span and spontaneous tumor development.

The mean life span of the control group was 709 days in males and 711 days in females, similar to the 623–735 days reported by Nakazawa *et al*.[34] Moreover, the tumor-free animals were observed to have a shorter life span compared with the tumor-bearing animals. The aging process predisposes cells to accumulate DNA mutations in oncogenes or tumor suppressor genes,[35] so the frequency of tumors increases with time, and most tumors arise in the last quarter of life.[36] In accordance with this fact, the majority of mammary or skin/subcutaneous tumors in our present study were observed to occur after the age of 500 days (16 months).

768The results of survival analysis suggested that MCPs could exert a significant effect on the mean life span of the long-lived subpopulation. The significant life-prolonging effects on the tumor-free animals and tumor-bearing animals indirectly reflected that MCPs might prevent the development of age-related non-neoplastic lesions as well as neoplastic lesions. In the opinion of Anisimov,[22] the type of geroprotectors, which decrease the mortality rate in the long-lived individuals and increase the maximum life span, was thought to slow down the process of aging and the development of age-related pathology.

The age-related spontaneous tumor incidences, with 80.0% in control females and 65.0% in control males, were near but slightly less than the frequencies of 87.0–95.8% and 70.0–76.7% reported for aged female and male S-D rats, respectively.[37] In the present study, pituitary gland adenomas and mammary adenomas developed most frequently in the control rats, which corresponded to the oncological characteristics of S-D rats.[38,39] Decreasing tendencies were observed in the incidences of pituitary gland and mammary gland tumors in MCP-treated rats, while the significance of these findings still must be confirmed in a larger sample size.

The decreased size and increased growth time of the palpable tumors, including mammary tumors and skin/subcutaneous tumors, implied that administration of MCPs might inhibit the development and the growth rate of existing tumors. In accordance with the result, the decreased frequencies of death cause for mammary or skin/subcutaneous benign tumors in MCP-treated groups indicated the inhibitory effect of treatment with MCPs on the tumor development or lethality. Therefore, the influence of MCPs on total tumor incidence and malignancy rate as well as tumor development might account for the decreased frequency of death due to neoplasm in MCP-treated groups. As a whole, certain inhibitory effects of MCPs on the spontaneous carcinogenesis in S-D rats of both sexes were revealed in our study, which contributed to the longevity extension of rats and proved the safety of lifelong oral administration of MCPs. In accordance with the result, previous studies demonstrated the synthetic collagen-like polypeptides containing glycine, proline, and hydroxyproline are the functional factors that inhibit the tumor growth and the development of tumor metastasis.[40–42] In addition, our study indicated that a higher percentage of rats in the MCP-treated groups spontaneously died of age-related non-neoplastic diseases compared with the vehicle-treated control group. This finding may be due to the longer life span of MCP-treated rats compared with the controls.

It must be noted that the effects of MCPs on survival and spontaneous tumor development were not associated with its impact on the body weight of the animals and the amounts of food consumed by them. No reduction in food consumption or body weight of MCP-treated rats was indicated during the observation. Only at the age of 3 months was a decrease in food consumption recorded in female MCP-treated groups, which was thought to be incidental for the absence of dose-related effect. Life span and aging-related diseases (including cancer) are positively related with body weight increase induced by excessive energy consumption.[43] The effect of caloric restriction on life span extension is mainly due to attenuating the rate of age-related increase in reactive oxygen species production and reactive oxygen species-associated damage to biomacromolecules such as proteins, lipids, and DNA.[44–46] Thus, oxidative stress is considered to be responsible for part of the aging process through variations in reactive oxygen species generation, reactive oxygen species elimination, or both.[47]

In the present study, the serum oxidative status during aging was investigated. Consistent with other reports,[48,49] the levels of MDA equivalents (TBARS), the primary product of lipid peroxidation, increased with age in all groups. Moreover, the activity of antioxidant system enzymes, including SOD and GSH-PX, decreased with age in our present study. In our experiment, MCPs were observed to exert a considerable effect on the age-related oxidative status, namely, the inhibition on the age-related decrease in the activity of antioxidant enzymes and age-related increases in the levels of TBARS. In agreement with the results, our previous study suggested administration of 0.22%, 0.44%, and 1.32% MCPs for 3 months could dose-dependently increase the activity of SOD and GSH-PX and decrease the level of TBARS in aged C57BL/6J mice.[50]

In a previous study of our laboratory, MCPs were suggested to exhibit significant radical scavenging activity and anti-lipid peroxidation activity, which were assessed with a 2,2-diphenyl-1-picrylhydrazyl radical scavenging assay (the 50% inhibitory concentration value of MCPs to scavenge 2,2-diphenyl-1-picrylhydrazyl radical was 20.719 mg/mL) and linoleic acid peroxidation system (10 mg/mL MCPs inhibited 58.69% linoleic acid oxidation after a 2-hour incubation), respectively.[51,52] Therefore, MCPs was presumed to react with radicals in the system and thereby directly inhibit the formation of lipid peroxidation product, such as MDA. In line with our results, in a study by Mendis *et al.*,[20] the peptide purified from enzymatically prepared fish skin gelatin hydrolysate was verified to increase the antioxidative enzyme levels in cultured human hepatoma cells with the possible mechanism involved in maintaining the redox balance in the cellular environment. However, the specific molecular mechanism underlying the induction of antioxidative enzymes by MCPs is still not elucidated.

As we know, MDA is a potentially important contributor to DNA damage and mutation and is verified to be carcinogenic in rats.[53] Disturbances in the oxidative status by weakened defense systems and increased peroxides can lead to age-related degenerative diseases as well as cancer.[54] Moreover, lifelong reduction in SOD activity was suggested to result in increased DNA damage and higher incidences of cancer.[55] It is confirmed that antioxidants or the free radical scavengers are shown to be the inhibitors at both initiation and during the promotion/transformation stage of carcinogenesis by protecting cells against oxidative

769damage.[56] For this reason, we suggest that the protection by MCPs against age-related oxidative stress may contribute to the effect on inhibiting the incidence of spontaneous tumors and prolonging the life span in S-D rats.

In conclusion, the data presented indicate that MCPs administered throughout the life span have significant influence on longevity and spontaneous tumor incidence in S-D rats. Moreover, antioxidative activity of MCPs might be involved in the mechanism. Further research is needed to elucidate the specific functional components of MCPs as well as the molecular and physiological mechanism of life-prolonging and anticancer effects of MCPs.

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AUTHOR DISCLOSURE STATEMENT

No competing financial interests exist.

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