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Quantitative Study of Neurons with Intracytoplasmic Pigments in Dorsal Root Ganglia
—— Atomic Bomb and Aging ——

Masao KISHIKAWA, Mika MATSUMOTO, Masachika ISEKI, Hisayoshi KONDO*, Osamu SHIMADA**, Nobuo TSUDA** and Hideharu FUJII***

*Department of Pathology & *Department of Statistics, Scientific Data Center of Atomic Bomb Disater, and **Pathology Division, Department of Laboratory Medicine, Nagasaki University School of Medicine, and ***Department of Pathology, National Nagasaki Central Hospital*

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ABSTRACT : Three females in their fifties and five males in their seventies, all of whom had been exposed to the Nagasaki atomic bomb explosion in 1945, were investigated concerning the aging of neurons of the dorsal root ganglia. Through this study dealing with the frequency of neurons with intracytoplasmic pigments, lipofuscin, neuromelanin, eosinophilic granules and neurons free of pigment, no significant difference in four kinds of neurons between the exposed persons and the nonexposed persons was detected in 50-59 year-old females. Contrariwise in the 70-79 year-old males, the frequency, as average but not as individual persons, was significantly higher in lipofuscin, lower in neuromelanin, and unchanged in eosinophilic granules and no pigment.

INTRODUCTION

There still remain conflicting opinions as to whether or not irradiation accelerates aging. It has been reported that lifespan of medical personnel is shortened by irradiation (SELTSER and SARTWELL[7] ; WARREN[12]) but almost all reports stating that ionizing radiation caused accelerated aging have been based on animal experiments (STORER and SANDERS[9] ; UPTON[10]).

The authors are carrying out continuing studies into neuronal aging with respect to three kinds of pigments, which are lipofuscin (LP), neuromelanin (NM) and eosinophilic granule (EG) as indices of aging (IKEYAMA *et al.*[1]; KISHIKAWA[2] ; KISHIKAWA *et al.*[3] ; SHINKAI *et al.*[8]). The present study deals with the frequency of neurons with intracytoplasmic pigments in the dorsal root ganglia in humans exposed to the Nagasaki atomic bomb explosion in 1945.

MATERIALS AND METHODS

The materials for this study were autopsy cases of individual who had been exposed to the Nagasaki atomic bomb explosion within 3km from the hypocenter, and from which dorsal root ganglia (DRG) specimens were obtained. Control cases were chosen with respect to similarity in age and the year of autopsy.

Address for correspondence : Masao Kishikawa, M. D., Department of Pathology, Scientific Data Center of Atomic Bomb Disaster, Nagasaki University School of Medicine, 12-4 Sakamoto-machi, Nagasaki 852, Japan

After preparation of hematoxylin-eosin stained specimens from the DRG of the cervical and lumbar regions for quantitative study, 250 cells with readily recognizable nuclei were chosen at random from each specimen. The neurons were counted by two observers (MK & MM), using a discussion-microscope which can be permitted to view by two investigators simultaneously. The neurons with different opinion were omitted from counting. The frequency (as average, not as individual persons) of the neurons with LP, NM, EG and neurons free of pigment was calculated. The control group consisted of age-matched cases not exposed to the atomic bomb explosion and autopsied at approximately the same time as the study group.

RESULTS

Three females in their fifties and five males in their seventies, all of whom had been exposed to the Nagasaki atomic bomb explosion within 3km from the hypocenter, were available for this study. Autopsies were performed during the 5-year period from 1978 to 1982 and DRG specimens were obtained from all cases (Table 1).

Table 1 Materials investigated

Case	Age	Sex	Age('45)	Exp. Status	Pathological diagnosis
AO1400	54	F	23	—	Carci.,breast
AO1417	58	F	28	—	Pulmonary fibrosis
AO2102	57	F	20	—	Hepatocellular carci.
AO2109	58	F	31	—	Diabetes mellitus
AO2133	57	F	20	—	Hepatocellular carci.
12866	51	F	17	2.0km	Aplastic anemia
AO1950	51	F	15	1.3km	Hepatocellular carci.
12902	55	F	20	2.6km	Adenocarci.,colon
AO1425	76	M	45	—	Malignant lymphoma
AO1445	77	M	46	—	Adenocarci.,stomach
AO1453	70	M	39	—	Cerebral hemorrhage
AO1458	79	M	47	—	Adenocarci.,gallbladder
12718	75	M	41	—	Malignant lymphoma
13149	73	M	37	2.0km	Myocardial fibrosis
12714	73	M	39	2.0km	Dissecting aneurysm
12618	78	M	45	1.2km	Emphysema
13532	71	M	34	3.0km	Malig. nephrosclerosis
12633	79	M	46	3.0km	Myocardial infarction

As is usually true, the three kinds of pigment were often intermingled in the same neuron so that the total frequency of their appearance exceeded 100% (Table 2). The frequency of LP

Table 2 Frequency of DRG neurons with intracytoplasmic pigments

		Cervical DRG		Lumbar DRG		Cervical & Lumbar DRG	
		50yr. F.	70yr. M.	50yr. F.	70yr. M.	50yr. F.	70yr. M.
LP	Cont	60.84	66.74	68.60	59.68	64.72	63.21
		10.60	10.60	9.70	4.84	10.87	8.97
	Exp	64.80	69.76	73.34	72.43	69.07	71.10
		8.03	5.94	8.28	10.33	9.20	8.53
NM	Cont	6.62	10.30	3.32	18.98	4.97	14.64
		3.59	5.67	2.39	7.83	3.47	8.10
	Exp	5.20	9.12	8.09	7.53	6.64	8.33
		0.86	4.61	3.70	5.41	3.05	5.09
EG	Cont	35.08	40.52	41.65	33.72	38.73	37.12
		16.27	20.25	17.34	17.33	17.14	19.15
	Exp	42.40	42.00	39.53	46.63	40.97	44.31
		9.07	12.07	4.85	19.17	7.42	16.19
NO	Cont	32.23	22.76	27.00	20.93	29.61	21.85
		8.28	6.32	8.50	7.18	8.78	6.83
	Exp	29.73	21.60	21.71	19.78	25.72	20.69
		7.28	7.97	6.24	8.26	7.88	8.17

LP: lipofuscin, NM: neuromelanin, EG: eosinophilic granule, NO: no pigment found, Cont: control cases, Exp: exposed cases: The upper numeral shows mean value and the lower one shows standard deviation.

showed no statistically significant difference between the two 50-59 year-old (6th decade) female groups, although it tended to be slightly higher in the atomic bomb-exposed group (Fig. 1). Among the 70-79 year-old (8th decade) males, the frequency of LP was significantly higher in the atomic bomb-exposed group ($p<0.05$, Fig.1). With regard to NM, there was no significant difference in frequency between the two 50-59 year-old female groups, while the frequency was significantly lower in the atomic bomb-exposed 70-79 year-old male group than in the control group ($p<0.01$, Fig. 2). With regard to EG, there was no significant difference between the two 50-59 year-old female groups. A slightly higher tendency was observed in the 70-79 year-old atomic bomb-exposed male group, but the difference was not statistically significant (Fig. 3). The frequency of neurons without pigments

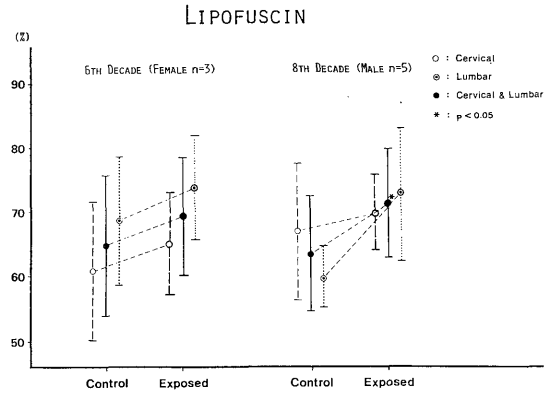

Fig. 1 LP-containing neurons. Among the 70-79 year-old males, the frequency was significantly higher in the exposed group.

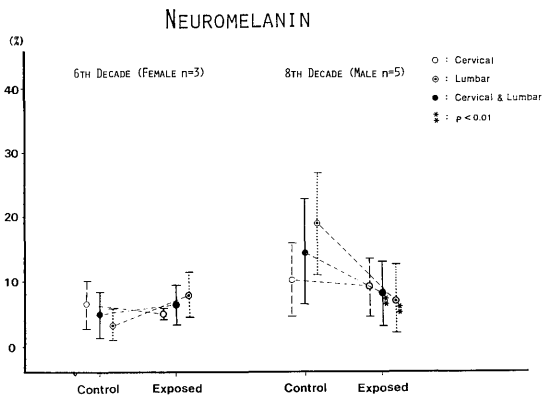

Fig. 2 NM-containing neurons. The frequency was significantly lower in the exposed 70-79 year-old males group.

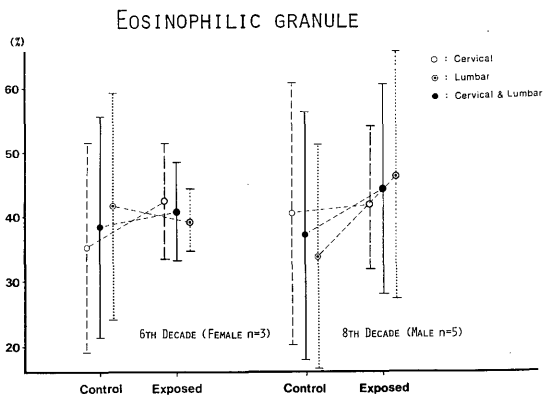

Fig. 3 EG-containing neurons. No significant difference was detected in both 50-59 year-old groups and 70-79 year-old groups.

was virtually the same in the 50-59 year-old females and 70-79 year-old males, and no significant difference was observed between the atomic bomb-exposed groups and the control groups (Fig. 4).

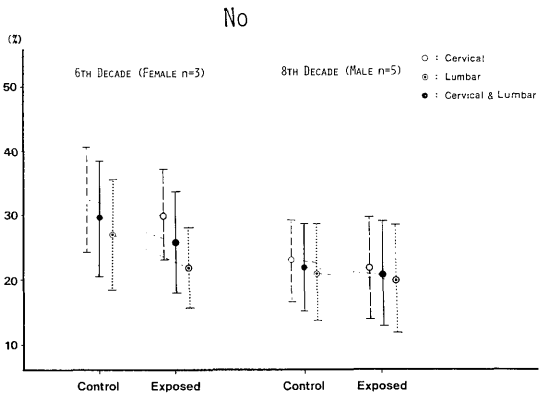

Fig. 4 Non-pigmented neurons. No significant difference was noticed in both 50-59 year-old groups and 70-79 year-old groups.

DISCUSSION

Although research concerning biological aging is being carried out through a wide range of animal experiments and studies on human materials, the mechanism of aging has not been elucidated. Irradiation damage has long been said to be one of the factors contributing to the aging of the biological organisms (STORER and SANDERS[9]; UPTON[10]). Some controversy, however, has resulted on the basis of animal experiments conducted to clarify the relationship between irradiation and aging (WALBURG[11]). Therefore, further investigation is definitely necessary, that is, studies to determine what indices are important in discussing the problem of aging. In most animal experiments to date, aging acceleration has been reported mainly in terms of shortened lifespan. It is obvious, however, that although death can be defined as the final end point of the aging process, death is not necessarily the result of aging. In particular, various controversial points arise when the above theory is applied simply to the human body.

Neurons and myocardial cells, as post-mitotic

cells, are ideal materials for the morphological investigation of aging. NAMIKI *et al*[6] studied post-mortem brain specimens from people exposed to the Hiroshima and Nagasaki atomic bomb explosions. According to their data there was no significant difference in the incidence of senile brain changes except for the fact that the incidence of Alzheimer's neurofibrillary tangles in the proximally exposed group, who had been exposed to the atomic bomb blast within a distance of 2.0km from the hypocenter, was very slightly higher. But there was no description of the intraneuronal pigmented granules such as LP, because only senile plaques and Alzheimer's neurofibrillary tangles were investigated(NAMIKI *et al.*[6]).

The authors of the present study are conducting ongoing investigations using LP and other intracytoplasmic pigments of neuron as indices for nerve cell aging (IKEYAMA *et al.*[1]; KISHIKAWA[2]; KISHIKAWA *et al.*[3]). It is difficult to evaluate the data of cerebrum, because the findings are variegated by region and the depth of paraffin section. On the contrary, it is relatively invariable in data of DRG. The DRG are easily available to be investigated even if the brain autopsy is not permitted. But studies concerning DRG have been scarce. The present study is the only investigation concerning DRG-neurons from the autopsy cases of atomic bomb-exposed subjects. Through this investigation in the 50-59 year-old exposed females, no significant difference in LP, NM, EG or no pigment was detected even though there was a slightly higher frequency of LP-containing neurons. Contrarily, a significant increase of neurons with LP and a reduction of neurons with NM were observed in 70-79 year-old atomic bomb-exposed males. The high frequency of LP-containing neurons and a reduction of neurons with NM in this group were a very interesting discovery. Usually, the frequency of neurons with LP increases with age but reaches a peak in the sixth or seventh decade of life (KISHIKAWA *et al.*[3]), while the NM does not usually show age-related variation (IKEYAMA *et al.*[1]; KISHIKAWA[2]). In the present study the control group showed this usual LP pattern. The fact that the difference was insignificant in the 50-59 year-old exposed females may probably be attributed to the very small number of exposed cases being only 3. It seems impossible, therefore, to completely deny the relationship between irradiation and elevated frequencies of LP-containing neurons in the 50-59 year-old cases. In experiments with rats, the cerebellar Purkinje cells, which are usually very low in LP, showed an increase of that pigment when exposed to a single high dose of radiation (3Gy), while no significant difference from the control group was observed when the rats were exposed to a dose of only 1.5Gy (SHINKAI *et al.*[8]). In atomic bomb-exposed humans, therefore, the dose of radiation should be considered along with the distance from the hypocenter (the latter was the only criterion in the present study), otherwise the results of investigations cannot be claimed to be conclusive. According to the estimated radiation dose (T65D : MILTON and SHOHOJI[5]), the radiation dose in our cases varied from 0.5rad to about 121rad. But there were too few cases in the exposed groups to determine a relationship between atomic bomb exposure and acceleration of senescence. In recent years, there has been an attempt to clarify the doses of atomic bomb energy exploded in Hiroshima and Nagasaki. We should, therefore, reexamine these data using the new information (DS86 : Dose System '86).

There are several other important points that must be included in the discussion on aging. One is the necessity, when processing data statistically, to match specimens not only according to sex and age but also to the calendar year when the victims were autopsied, since unlike experimental animals, the morphological characteristics of the individual human is influenced greatly by numerous factors such as living environment, diet, amount of exercise, concurrent illnesses, etc. The present study focused exclusively on age and sex-matched cases that were autopsied around the same period, but there were very few atomic bomb-exposed cases from which DRG specimens had been obtained and, as a result, it was necessary to restrict the investigation to females in the sixth decade and males in the eighth decade of life.

It remains extremely difficult to explain the fact that, although NM dose not usually show a correlation with age (IKEYAMA *et al.*[1]), the frequency in the 70-79 year-old atomic bomb-

exposed males was significantly lower than that in the control cases. It is thought that an intimate relationship, ultrastructural and otherwise, exists between LP and NM (KISHIKAWA[2]; LIEBERMAN[4]), but at present it is unknown whether or not there is a close mutual connection between the high frequency of LP-containing neurons and low frequency of the neurons with NM observed in this study.

To conclude, even though the results of this study may clarify the causal relation between the atomic bomb exposure and the high frequency of neurons with the age-related pigment, this study still fail to clarify the causal relation between the atomic bomb exposure and the acceleration of senescence. Because, the concept that LP is the age-related pigment is generally acceptable, however, it does not always mean directly that the frequency of LP-containing neurons increase in proportion to the individua l senescence-acceleration.

Like LP, EG shows a correlation with age (IKEYAMA *et al.*[1]; KISHIKAWA[2]; KISHIKAWA *et al.*[3]), but in the present study no significant difference was observed between the atomic bomb-exposed groups and the control groups. Further investigations are necessary to determine whether or not this difference is related to the mitochondrial origin of EG (KISHIKAWA[2]).

Materials and age indices in this study differ from those in the report by NAMIKI *et al.*[6] and so it might be impossible to compare the findings in the two studies. In the future, however, it will be necessary to discuss the correlation between the length of time after atomic bomb exposure and changes in age acceleration in the cases used in this study and other cases autopsied around the same period by means of a method similar to that employed by NAMIKI *et al.*[6].

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