Mechanisms of Ageing and Development, 42 (1988) 129—138
Elsevier Scientific Publishers Ireland Ltd.

EFFECT OF LIFETIME ADMINISTRATION OF DIMETHYLAMINOETHANOL ON LONGEVITY, AGING CHANGES, AND CRYPTOGENIC NEOPLASMS IN C3H MICE

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(Received June 5th, 1987)
(Revision received September 29th, 1987)

SUMMARY

The effects of lifetime treatment with dimethylaminoethanol on longevity and cryptogenic neoplasm formation were studied in females of two mouse sub-lines, the C3H/HeN which carries a germinal mammary tumor provirus and the C3H/HeJ(+) which also carries the exogenous mammary tumor virus. Administration in the drinking water of 10 mM dimethylaminoethanol to the C3H/HeN mice or 15 mM to the C3H/HeJ(+) mice did not result in significant differences between treated and untreated groups in average survival. No changes in age-related organ structure or morphology were observed with dimethylaminoethanol treatment, except for an apparent decrease in the amount of lipofuscin in the liver judged in histological sections. Among untreated C3H/HeJ(+) females, 89% developed neoplasms of the mammary gland, ovary, liver, lung and reticuloendothelial system, while the incidence was 88% in the treated mice. In C3H/HeN females, neoplasms of the mammary gland, ovary, liver, lung and lymphatic system occurred in 57% and in 60% of treated mice. Also, there was no statistically significant difference between control and treated animals in the age of onset or the type of specific neoplasms. Dimethylaminoethanol did not induce any neoplasms.

Key Words: Dimethylaminoethanol; Aging; Longevity; neoplasms

INTRODUCTION

2-Dimethylaminoethanol (DMAE) is a synthetic analog of choline which has been

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0047-6374/88/$03.50
Printed and Published in Ireland

used in humans to treat central nervous system disorders believed to be associated with hypofunction of cholinergic neurons [1—3] and the acetamidobenzoate salt is commonly used in the treatment of learning and behavior problems and hyperkinetic behavior [3]. DMAE caused central stimulatory effects in mice possibly as a result of conversion to choline and then to acetylcholine in the brain [4].

DMAE is formed in addition to *p*-chlorophenoxyacetic acid from aminoethyl-*p*-chlorophenoxy-acetate in aqueous solution [5]. The latter agent, also known as centrophenoxine or mechlofenoxate, is effective in reducing age-related changes such as lipofuscin accumulation in the brain [6,7]. When used in lifetime experiments, DMAE or centrophenoxine were reported to prolong survival significantly in several animal species [8—10].

The aging process has been postulated to involve alterations in cell membranes [11—13]. Choline is essential in the formation of cell membranes [14]. Published results suggest that DMAE inhibits choline metabolism in peripheral tissues [15]. DMAE elicited an increase in the concentration of choline in the blood of rodents and humans and in the rate of turnover of free choline in the blood [16].

Oxygen free radicals occur in biological systems [17] and have been implicated as etiological factors in several biological phenomena such as mutagenesis, carcinogenesis and aging [18]. One membrane hypothesis of aging attributes a role to free radicals in the alteration of cell membrane structure and function [11—13]. The hydroxyl free radical-scavenging property of DMAE is firmly established [19], supporting the possibility that it could retard aging by protecting cell membranes.

Antioxidants have been suggested to have cancer inhibitory properties [20—22], although, synthetic ones, like many other inhibitors, usually function as inhibitors of carcinogen-metabolizing enzymes or inducers of detoxifying systems [23]. Nevertheless, naturally occurring antioxidants affect cell growth and differentiation [24] and, some, such as vitamin E and ascorbic acid, have inhibited carcinogenesis in experimental studies [25,26]. Therefore, it is possible that DMAE might have anticancer activity. In support of this, it has been reported that centrophenoxine enhanced the killing effect of cytostatic drugs in tumor cell cultures [27]. Perhaps also relevant, choline deficiency sensitizes animals to low levels of liver carcinogens, reduces the latent period for neoplasm development and increases the incidence of carcinogen-induced neoplasms [28—30].

There is, however, reason to suspect that DMAE might influence cancer development differently. Structurally related di- and triaminoethanols found in cutting fluids, pesticides and cosmetics [31] can give rise to *N*-nitrosodiethanolamine (NDELA) through nitrosation resulting from nitrite or nitrous oxide [31,32]. NDELA is a potent carcinogen producing mainly hepatocellular carcinomas in rats [31,33,34], as well as epithelial neoplasms of the nasal cavity and trachea in hamsters [31,35,36]. If DMAE were similarly nitrosated, the resulting nitrosamine might be carcinogenic.

Based upon these considerations, the present study was undertaken to determine

the effect of DMAE on aging and lifespan of female mice of two sub-lines and to assess whether it reduced their cryptogenic neoplasm incidence or, conversely, induced neoplasms. The study involved female C3H/HeN mice which carry a dominantly expressed germinal mammary tumor provirus, Mtv-1 and develop a moderately high incidence of tumor appearing late in life and female C3H/HeJ(+) mice which also carry the exogenous milk transmitted mammary tumor virus causing a high and early incidence of mammary tumors [37].

MATERIALS AND METHODS

Animals

Mice were obtained from the National Institutes of Health Bethesda, MD and maintained in the research animal facility of the Naylor Dana Institute under the supervision of J. Silverman, D.V.M. The facility, which operates on a clean/dirty corridor system, was accredited by the American Association for the Accredition of Laboratory Animal Care. The care of animals conformed to the Guide for the Care and Use of Laboratory Animals (NIH-78-23).

Female C3H/HeN mice (groups 1 and 2), with the Mtv-1 provirus and female C3H/HeJ(+) mice, which also carry the exogenous murine mammary tumor virus (groups 3 and 4) were obtained at 4 weeks of age. They were housed 5/10.5 × 19.5 × 8″polycarbonate cage. The bedding used was heat-treated hardwood chips. The basal diet was NIH-07 (Zeigler Bros., Gardners, PA), which was freely available. Cages and food were changed three times a week. Tap water was provided in bottles with metal sippers, so that DMAE could be given in water to some groups. Animals were housed in a temperature (21 °C ± 1°) and humidity (50 ± 10%) controlled room with a minimum of 14 air changes/hour. The rooms were maintained on a 12-h light/dark cycle.

Chemicals and Administration

DMAE (Sigma Chemical Company, St. Louis, MO) was administered in the drinking water. Solutions of 10 mM (group 2) and 15 mM (group 4) were prepared by adding 8.9 and 13.4 g DMAE to 10 l of tap water, respectively. The solutions were neutralized to pH 7 using hydrochloric acid and kept refrigerated until use. The drinking water containing DMAE was given in amber glass bottles and made freely available.

Experimental Design

The study consisted of two separate experiments. In the first, 120 female C3H/HeN mice were divided into group 1, 60 controls, and group 2, 60 mice given 10 mM DMAE in drinking water. In the second experiment, two groups of 50 female C3H/HeJ(+) mice were allocated to group 3, controls and group 4 which received 15 mM DMAE in drinking water. The treatments were given for the durations of the experiments, 105 weeks for groups 1 and 2 and 123 weeks for groups 3 and 4.

The animals were observed regularly and notations made of all gross changes including palpable mammary nodules. The animals were weighed weekly. They were allowed to die of natural causes or killed when moribund. All animals were autopsied except those that died between observation times and were cannibalized or decomposed. These were not included in the effective number of animals. At autopsy all abnormal organs were described and sections were taken for histological examination. In addition, sections were taken from all parenchymatous organs. The specimens were fixed in buffered formalin. Tissue slices were embedded in paraffin and sections were prepared in the Histopathology Laboratory supervised by A. Rivenson, M.D. Hematoxylin-eosin stain was used as well as other stains when needed.

Statistical analyses were performed using the χ^2 test with one degree of freedom.

RESULTS

The dose of DMAE used in the first study was chosen to avoid toxicity. As shown in Fig. 1, lifetime administration to female C3H/HeN mice of 10 mM DMAE in the drinking water had no effect on the initial weight gain or mature body weights in treated mice. Therefore, in the second study in C3H/HeJ(+) female mice, a 50% higher dose, i.e. 15 mM was used. This also did not affect body weights (Fig. 1).

The survival of DMAE-treated C3H/HeN mice (group 2) or C3H/HeJ(+) mice (group 4) as compared to controls (groups 1 and 3) was not appreciably affected (Fig. 2). If anything, in treated groups, a slightly higher mortality was seen in older animals.

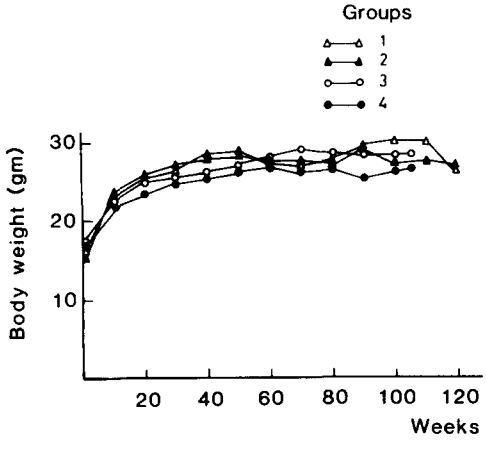

Fig. 1. Average weight of mice treated with dimethylaminoethanol and controls. Group 1, C3H/HeN control mice; 2, DMAE treated C3H/HeN mice; 3, untreated C3H/HeJ(+) mice; and group 4, C3H/HeJ(+) mice receiving DMAE for life.

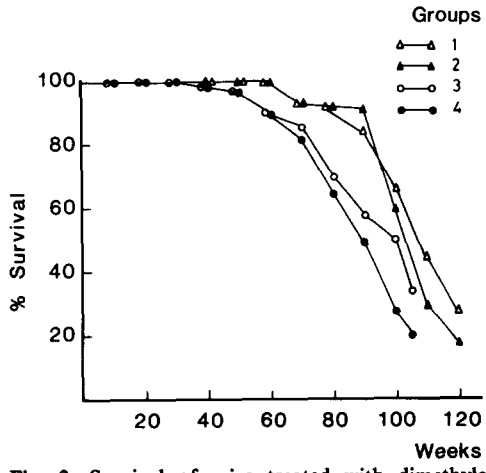

Fig. 2. Survival of mice treated with dimethylaminoethanol and controls. Group 1, C3H/HeN control mice; 2, DMAE treated C3H/HeN mice; 3, untreated C3H/HeJ(+) mice; and group 4, C3H/HeJ(+) mice receiving DMAE for life.

DMAE treatment did not induce any notable changes in the structure or appearance of different organs or in their microscopical morphology using conventional methods. Only the extent of lipofuscin appeared less distinct in the livers in DMAE-treated groups 2 and 4, when evaluated in hematoxylin and eosin stained sections, but this could not be quantified.

A large number of neoplasms developed in all groups (Table 1) and the influence of the exogenous mammary tumor virus in the C3H/HeJ(+) mice was evident from the high incidence of mammary tumors (Table II). The number of neoplasms in most organs was similar in the control and treated animals (Table II). However, in DMAE-treated mice a possible slight, though not statistically significant, decrease in the incidence of mammary neoplasms was observed, i.e. 16% in treated C3H/HeN mice *vs.* 24% in controls and 68% in treated C3H/HeJ(+) mice *vs.* 73% in controls. Histological study of the neoplasms in the different groups did not show

TABLE I

NUMBER OF NEOPLASM-BEARING ANIMALS AND NEOPLASMS IN FEMALE MICE EXPOSED TO DIMETHYLAMINOETHANOL AND CONTROLS

Group	*Exposure*	*Subline*	*Initial No.*	*Effective No.*	*No. Bearing a Tumor*	*Total Neo-plasms*
1	None	C3H/HeN	60	58	33	49
2	DMAE	C3H/HeN	60	50	30	39
3	None	C3H/HeJ(+)	50	44	39	57
4	DMAE	C3H/HeJ(+)	50	40	35	53

TABLE II

TYPES OF NEOPLASMS OCCURRING IN DIMETHYLAMINOETHANOL TREATED FEMALE MICE AND CONTROLS

Group	*Subline*	*Exposure*	*Effective No.*	*Mammary Carcinoma*	*Lymphoma*	*Liver Neoplasm*	*Lung Neoplasm*	*Hemangioma*	*Ovaries*			*Other*
									Granulosa cell tumor	*Tubular Adenoma*	*Cyst*	
1	C3H/HeN	None	58	14	4	6	7	1	4	6	33	7[a]
2	C3H/HeN	DMAE	50	8[e]	8	7	4	1	4	3	22	4[b]
3	C3H/HeJ(+)	None	44	32	0	4	3	4	2	9	35	3[c]
4	C3H/HeJ(+)	DMAE	40	27[f]	1	5	1	4	3	5	27	7[d]

[a]2 ovarian luteomas, 1 cervical polyp, 1 endometrial polyp, 1 uterine adenocarcinoma, 2 adrenal tumors.
[b]1 ovarian teratocarcinoma, 2 subcutaneous sarcomas, 1 adrenal tumor.
[c]2 luteomas; 1 endometrial adenocarcinoma.
[d]2 stomach squamous cell carcinomas, 1 duodenal adenocarcinoma, 1 renal carcinoma, 3 subcutaneous sarcomas.
[e]Not different from group 1, $P = 0.42$.
[f]Not different from group 3, $P = 0.776$.

effects related to DMAE in either mouse strain. Mammary neoplasms were mostly well-differentiated solid adenocarcinomas in DMAE-treated mice as well as in untreated animals. Liver neoplasms, adenomas and hepatocellular carcinomas, were morphologically similar in both treated and untreated groups. Hemangiomas were present in several tissues, i.e. liver, subcutaneous tissues and lymph nodes in DMAE-treated mice as well as controls, although the total number was low. Lung adenomas were nodular lesions comprised of cuboidal cells, which were similar in DMAE-treated animals and controls. Neoplasms of the genital system were numerous in all groups. These included mainly ovarian neoplasms, granulosa cell tumors, tubular adenomas, and a few luteomas as well as a few neoplasms of the uterus and cervix. Ovarian cysts, were numerous, 117 in total. Most were of the simple type with low flattened epithelium, only a few cystadenomas of serous or mucinous types were found. Cystic ovarian follicles were common, apparently as a result of failure of normal luteinization of Graafian follicles.

DISCUSSION

Administration of DMAE in drinking water to two sublines of female mice for lifetime did not have a significant effect on longevity. Previous reports on long-term administration of DMAE described an increased survival of treated strain A and Swiss-Webster mice [20,38,39]. These other experiments, however, used considerably higher doses, i.e. 1%/w or 2%/w compared to 0.09 to 0.13% in this study. Also, in these other studies, a sub-optimal survival of the animals may have provided the circumstances, as discussed by Kohn [40], for DMAE to increase survival.

Aging has been suggested to be due in part to the action of endogenously produced free radicals, [41]. In one test of this hypothesis [38], mice were given various free radical inhibitors in their diet; some of the treated groups were found to live about 20% longer than the controls. In another study, the survival time of mice was prolonged by hydroxylamine [20], as well as other reducing agents [39]. Cell membrane degradation from free radicals has been proposed as a prime mechanism of aging [13,17] and results from studies with centrophenoxine have been used in support of this concept [42,43]. Subsequent studies, however, have shown that antioxidants are effective only when survival is sub-optimal [40] and do not increase lifespan beyond that of controls living under optimal conditions. We have made a similar observation with hydroxylamine [44].

One of the best morphological indicators of aging is lipofuscin pigment [6,7]. This age pigment is formed by oxidative polymerization of lipids, probably largely mitochondrial, and proteins [11,13,17]. The tissue accumulation of lipofuscin can be slowed by antioxidants [45]. In the present study, a decrease in histologically detected lipofuscin pigment in the liver seemed to occur with DMAE treatment. In other studies, centrophenoxine-treated animals also showed a notable reduction of

lipofusion pigments in most parts of the central nervous system [6,7] which may be attributable mainly to the DMAE released. The degree of reduction was largely a function of the duration of treatment. In the present study, the reduction of lipofuscin accumulation was not associated with enhanced longevity.

Alteration in cellular membranes may play an important role in neoplasm formation [46,47]. DMAE is the immediate precursor of choline in the biosynthesis and repair of cellular membranes [4,15,16]. Choline deficiency has increased carcinogen-induced tumor formation, either by sensitizing the animal to low levels of carcinogens or by exerting a promotion-like effect [28—30]. Apparently, the level of choline in the diets used in the present studies did not influence the incidence of cryptogenic neoplasms since presumably raising the level by DMAE administration failed to produce a significant reduction in the occurrence of tumors.

The antioxidant properties of DMAE [19] may also endow the compound with antineoplastic capabilities such as reported for other antioxidants [20,21,25,26]. In mice innoculated with the Ehrlich ascites tumor, DMAE treatment produced a 20% extension of the survival time [39], while a number of other antioxidants, including cysteine hydrochloride and 2-mercapto-ethylamine hydrochloride, were not effective. However, in this study, DMAE did not inhibit the formation of most neoplasms, although the number of mammary neoplasms tended to be lower in treated groups. This study also provides information on the lack of carcinogenicity of DMAE. DMAE was given for lifetime with no increase of any type of neoplasm in either strain. Although the study was limited to female mice and the doses used were not maximally-tolerated doses, since toxicity was being avoided, the finding of no increase in neoplasms at least suggests that DMAE was not a potent carcinogen under the present conditions. This is perhaps of relevance to the fairly common environmental occurrence of DMAE at low levels i.e. the use as an emulsifier for cosmetics [48], in pesticides [49] in synthetic cutting fluids [50] and in tobacco [51].

ACKNOWLEDGEMENTS

We thank P. Radok for assistance with care and treatment of animals, Clare Mahan for statistical analysis and C. Meyer for preparation of histological material. The manuscript was typed by Mrs. T. Seppell. The studies were carried out at the Naylor Dana Institute, American Health Foundation, Valhalla, NY 10595.

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