The mitigating effect of dietary antioxidants on chemically-induced carcinogenesis[1]

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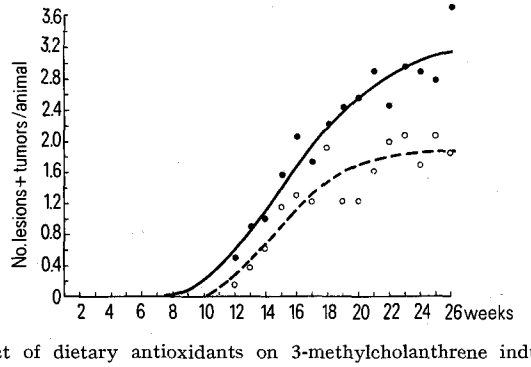
Summary. The effect of a dietary antioxidant mixture on 3-methylcholanthrene mediated carcinogenesis in hairless mice was investigated. The antioxidant mixture significantly reduced the frequency of premalignant lesions and their subsequent development into tumors. The similarities in response of chemical and UV light-carcinogenesis to these antioxidants suggest some congruity in the mechanisms of the carcinogenic process.

Harman demonstrated that certain antioxidants produced a marked decrease in incidence of spontaneous cancer development in strains of mice so predisposed[2,3]. Since that time numerous antioxidants have been shown to effectively inhibit the effects of chemical carcinogens[4–8]. Whereas literature is accruing that attests to antioxidants' effectiveness in inhibiting chemical carcinogenesis, little is known of their mode of action. Recently, we demonstrated that a mixture of antioxidants, incorporated into the diet of hairless mice, was effective in protecting against UV light (UVL)-induced formation of cholesterol α-oxide, a possible biogenic carcinogen[9]. Subsequent studies indicated that this same antioxidant mixture was effective in suppressing UVL-mediated tumor initiation and development[10,11]. Cholesterol α-oxide is a UVL-induced photoproduct of cholesterol – a natural and richly abundant constituent of skin. This compound could exert its effects(s) in a fashion similar to other chemical carcinogens. Therefore, we sought to determine whether dietary antioxidants were equally effective against the effects of a topically-applied chemical carcinogen as against those induced by UVL and thus whether some congruity might exist between the mechanism(s) of these 2 carcinogenic agents.

Materials and methods. 2 groups of albino, hairless mice (hrhr), 22–25 g, were selected for the study. One group of 18 animals (control) was maintained on a balanced laboratery meal (Wayne Lab-blox for Hamsters and Mice, Allied Mills, Chicago, Illinois). The other group of 18 animals received the same meal supplemented with a 2% (w/w) antioxidant mixture composed of the following: 1.2% ascorbic acid, 0.5% butylated hydroxytoluene, 0.2% DL-α-tocopherol (250 IU vit. E/g) and 0.1% glutathione (reduced form). After 2 weeks, the dorsal median of each animal, from the shoulder to hip, was painted once with 100 µl of 3% 3-methylcholanthrene (MCA) in acetone. 100 µl of 0.5% croton oil in acetone was applied once weekly for 15 weeks. Animals were examined regularly for the occurrence of chemically-induced lesions. Raised lesions of approximately 1 mm diameter, morphologically resembling actinic keratoses, were taken as a biological end-point in evaluating the chemicals' effect. These lesions were histologically diagnosed as pre-malignant. Tumors were diagnosed histologically as papillomas. The percent of animals bearing tumors or lesions, number of lesions per animal, and number of lesions and tumors per animal were the parameters chosen for evaluation. Comparisons of these parameters from both groups of animals at weekly intervals were statistically evaluated using the Wilcoxon Rank Sum Test. Many of the papillomas eventually developed into squamous cell carcinomas, however, this occurred eight months beyond the experimental period.

Results and discussion. As early as week 12, the number of lesions per animal was strikingly higher in the control animals (table). This difference is statistically significant at weeks 19 ($p<0.01$), 24 ($p<0.05$), 26 ($p<0.05$) and thereafter. Tumors appeared in both groups of animals after 14 weeks of treatment. There was no significant difference in the tumor prevalence, i.e., percent of tumor-bearing animals. However, 33 weeks after treatment, tumor frequency (number of tumors per animal at each observation) in the control group was almost twice that of the antioxidant-supplemented group. Total number of lesions and tumors per animal was constantly higher in animals of the control group. This is graphically represented in the figure where number of lesions and tumors per animal is compared over the first 26 weeks of the study. Results from this study suggest that antioxidants not only act upon the initiation of tumorogenesis but also affect the development of tumors from pre-malignant lesions upon chemical promotion.



Effect of dietary antioxidants on 3-methylcholanthrene induced lesions and tumors in hairless mice. Solid line represents animals on regular diet and the dotted line represents animals on antioxidant supplemented diet. 12 weeks after initiation of the study, 5 animals of the group on antioxidant supplemented diet were lost due to negligence in animal maintainance. The rest of animals in both groups survived the entire study period. The model used to approximate the curves is: $Y = P_1(1\text{-}e^{-P_2(X-P_3)^2})$ where $P_1 = 3.28$; $P_2 = 0.0092$; $P_3 = 7.14$ for the group on regular diet and $P_1 = 1.87$; $P_2 = 0.0206$; and $P_3 = 0.26$ for the group on antioxidant supplemented diet; X = time in weeks.

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Summation of the effects of dietary antioxidants on MCA induced cutaneous carcinogenesis

Diet	Parameters	Week 12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	33	37
Regular	Animals with tumors or lesions (%)	33	61	56	67	72	67	61	83	78	94	83	94	94	89	94	94	100
	Animals with tumors (%)	0	0	0	11	17	28	28	22	38	56	44	50	44	56	50	66	72
	No. lesions/animal	0.50	0.89	1.00	1.39	1.83	1.33	1.72	1.83	1.78	2.00	1.67	2.11	2.17	1.89	2.89	2.76	2.33
	No. tumors/animal	0.00	0.00	0.00	0.17	0.22	0.39	0.50	0.61	0.78	0.89	0.78	0.83	0.72	0.89	0.83	1.12	1.67
	No. lesions + tumors/animal	0.50	0.89	1.00	1.56	2.06	1.72	2.22	2.44	2.56	2.89	2.44	2.94	2.89	2.78	3.72	3.88	4.00
Special	Animals with tumors or lesions (%)	15	23	23	69	62	62	77	62	69	85	100	85	77	85	92	85	77
	Animals with tumors (%)	0	0	8	15	15	31	38	23	46	38	46	62	54	46	54	46	54
	No. lesions/animal	0.15	0.38	0.54	0.92	1.08	0.85	1.38	0.46	0.62	1.08	1.23	1.15	0.85	1.23	1.15	1.15	1.38
	No. tumors/animal	0.00	0.00	0.08	0.23	0.23	0.38	0.54	0.77	0.62	0.54	0.77	0.92	0.85	0.85	0.69	0.62	0.77
	No. lesions + tumors/animal	0.15	0.38	0.62	1.15	1.31	1.23	1.92	1.23	1.23	1.62	2.00	2.08	1.69	2.08	1.85	1.77	2.15

Free radicals have been implicated in a number of pathological conditions[12,13]. Increased levels of free-radicals and lipid peroxidation have been reported in skin following UVL-radiation[14,15], Many anticancer compounds exhibit free-radical inhibitory effects[16,17]. Indeed, some of these have recently been shown to inhibit chemically-induced skin carcinogenesis[18]. Whether free-radicals are involved in either UVL or chemically-induced skin carcinogenesis is yet unknown. However, the results of this study indicate that antioxidants are effective in allaying the deleterious effects of topically-applied 3-methylcholanthrene and suggest that there may be a confluence in the developmental steps of both chemical and UVL-induced carcinogenesis at which antioxidants act.

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The endocrine nature of the paraganglia of man

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Summary. Brightly fluorescent paraganglia were found in the retroperitoneal tissue of adult man. The histofluorescence properties of the paraganglia indicate the presence of tryptophyl peptides, which might be of endocrine importance.

The extra-adrenal catecholamine storing and syntethizing cells are widely distributed along and within the sympathetic and parasympathetic nervous system[1-3]. These cells, including the small, intensely fluorescent (SIF) cells of the sympathetic ganglia[4-7] are described as paraganglia (PG)[1-8].

The PG of man dominate during the fetal period[1,2], while only a few observations on their postnatal fate are available[1,9-11]. To elucidate the nature of the human adult PG, tissues obtained from vascular, gynaecological and urological surgery were analyzed systematically using the formaldehyde induced fluorescence (FIF) method[2,4] for catecholamines to trace the PG.

As acknowledged in the preliminary reports[12,13], brightly yellowish to orange fluorescent PG were regularly found embedded in the para-aortic and retroperitoneal connective tissue (figures 1 and 2). Microspectrofluorimetric recordings of the emission spectra of the fluorophore showed emission maximum at 480 nm typical to catecholamines (figure 3). The PG are considered as APUD cells[14] characterized by Pearse[15,16]. The 2 most important common characteristics of the APUD cell series are

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