

Effects of Aging on the Induction of Angiosarcoma

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Adult, Sprague-Dawley albino rats of four different ages (6, 18, 32 and 52 weeks) were exposed to 940 ppm vinyl chloride by inhalation for 24 weeks, 5 days/week, 7 hr/day. In each age group, there were 110 to 128 males and the same number of females. The similarly housed control group, which was not exposed to vinyl chloride, consisted of the same number of males and females in each age group. All animals that died spontaneously, or were sacrificed moribund, or were killed at scheduled times (3, 6 and 9 months after initial exposure) were autopsied. All organs were examined grossly, and several tissues from each animal were examined microscopically.

The older the rats were when they were first exposed, the greater the incidence of angiosarcomas. The incidences of angiosarcomas in the four age groups (from youngest to oldest) in the exposed males in the nonscheduled sacrifice groups were: 0/37 (0%); 0/44 (0%); 3/45 (6.7%); and 13/55 (24%). Similarly, for the females, these incidences were: 2/38 (5.3%); 7/47 (15%); 23/49 (47%); and 11/54 (20%). Most of the angiosarcomas were highly anaplastic, primary tumors in the livers that metastasized to the lungs. Only one angiosarcoma was seen in all the control rats; that occurred in subcutaneous tissue.

This study demonstrated that older adult animals and females are more susceptible to the angiosarcoma-inducing effects of vinyl chloride than young adult animals and males, respectively.

Introduction

Several epidemiological studies performed on occupational groups exposed to carcinogens have shown greater increased risks of cancer with increasing exposure durations. In addition, latent periods (time between initial exposure and increased risk of cancer) in these groups are commonly 10-30 years. These findings have been commonly attributed to greater total doses in those exposed for longer periods of time. However, there is one factor other than the total dose which could be responsible for contributing to the increased risk. That factor is

aging. It is equally reasonable to postulate that older adults are more susceptible to the carcinogenic effect of some chemicals than young adults. That is, for the same dose of carcinogen, the incidence of cancer might be higher and the latent period shorter in older adults than in younger adults.

It has been suggested that only older people should work in carcinogenic environments, since the commonly observed latent periods for cancer induction would exceed their life expectancies. If, however, the latent periods were shorter in older individuals, that approach would be counterproductive.

The purpose of this study was to determine whether or not older adult rats were more or less susceptible to the angiosarcoma-inducing effects of vinyl chloride than young adult rats. This experiment was not designed to test the susceptibility of sexually immature animals or to determine the lifetime effect of relatively short-term exposures.

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Materials and Methods

A total of 473 male and 478 female Sprague-Dawley rats were exposed to 948 ppm of vinyl chloride monomer in a single $14 \times 13 \times 10$ ft stainless steel walk-in chamber with a diffusion-screen false ceiling. Exposures were for 7 hr/day, 5 days/week for a mean duration of 24.5 weeks. An equal number of control male and female rats, kept in a similar exposure chamber, were exposed to conditioned outside air only. Air flow into the chambers was maintained at $300 \text{ ft}^3/\text{min}$. Air temperatures were kept at $75 \pm 3^\circ\text{F}$. The concentration of vinyl chloride in the chambers was initially monitored by gas chromatography and later with an ultraviolet photoionization analyzer, 7 times/day on each day of exposure.

The animals were individually housed in suspended stainless steel wire-mesh cages equipped with automatic watering taps. Water was provided *ad libitum*, but food (Wayne Lab Blox) was removed during exposures.

Four differently aged sets of rats of both sexes

were used in both the exposed and control groups. Their ages at initiation of exposure were 6, 17-18, 32-33 and 51-53 weeks. The numbers in each group and their disposition appear in Tables 1 and 2.

Because of the large number of animals, it was not practical to start exposures on all rats simultaneously. Consequently, the controls and exposed rats were divided into five squads, with proportionate representation of each age group and sex in each squad. Rats in each squad were assigned to the chambers over a 2-week period. Assignments of all rats to the chambers were completed throughout a 10-week period.

The original plan was to expose the rats for 12 months, but because of an epidemic of pneumonia among the exposed animals during the 28th week, the exposures were terminated 29 weeks after initiating exposures. As the results will show, that exposure period was adequate to demonstrate the differences in effects between groups.

The experiment consisted of two subsets. Animals scheduled for sacrifice at 3, 6 and 9 months constituted one subset, and animals that died or

Table 1. Number and disposition of rats exposed to vinyl chloride.

Group	Age at first exposure, weeks	Mean body weights at first exposure, g	Sex	Initial number of rats	Number of deaths and moribund sacrificed	Number at final sacrificed	Number sacrificed at 3, 6 and 9 months	Total number of rats autopsied
2	6	228	M	110	36	6	66	108
2	6	167	F	110	16	23	70	109
4	17-18	541	M	119	39	13	67	119
4	17-18	304	F	120	33	16	70	119
6	32-33	693	M	116	43	4	69	116
6	32-33	350	F	120	45	6	69	120
8	51-53	739	M	128	60	0	67	127
8	51-53	375	F	128	52	6	70	128
Totals			M	473	178	23	269	470
			F	478	146	51	279	476

Table 2. Number and disposition of control rats.

Group	Age at first exposure, weeks	Mean body weights at first exposure, g	Sex	Initial number of rats	Number of deaths and moribund sacrificed	Number at final sacrificed	Number sacrificed at 3, 6 and 9 months	Total number of rats autopsied
1	6	230	M	110	9	6	69	84
1	6	173	F	110	6	23	71	100
3	17-18	550	M	119	11	13	70	94
3	17-18	304	F	120	9	16	70	95
5	32-33	701	M	115	12	4	70	86
5	32-33	353	F	120	10	6	69	85
7	51-53	772	M	128	23	0	70	93
7	51-53	385	F	127	27	6	69	102
Totals			M	472	55	23	279	357
			F	477	52	51	279	382

were sacrificed moribund or were killed at the final sacrifice constituted the other subset. To keep these two subsets separate, the former will be referred to as the interim sacrifice group and the latter as the nonscheduled sacrifice group. When all the rats in one of the exposed groups had died, the remaining rats in all the other exposed groups were autopsied. This occurred 43 weeks after exposures began and is referred to as the final sacrifice. The number of control rats in each group autopsied at the final sacrifice were equal in number to the rats in each of their respective exposed groups.

At the interim sacrifices, blood was taken from each animal for hematological and clinical chemistry analyses, including measurements for albumin, gamma globulin, alkaline phosphatase, SGOT, SGPT, and γ -glutamyl transpeptidase.

At the autopsies all tissues were examined grossly and sections of liver, kidney, pancreas, spleen, adrenal, thyroid, pituitary, brain, gonads, prostate or uterus, urinary bladder, skin, sternum, paws, lungs, tracheobronchial lymph nodes, mesenteric lymph nodes, salivary gland, zymbal glands and any abnormal tissues from each rat were fixed in neutral buffered 10% formalin, embedded in Paraplast, slide-mounted, stained with hematoxylin and eosin and examined microscopically by a pathologist.

Results

No clinically or statistically significant differences in mean hematological and clinical chemistry measurements were detected between exposed and control groups of the same age and sex at any of the interim sacrifices.

The most frequently occurring tumor types in both sexes of exposed rats were liver angiosarcomas, pituitary adenomas and mammary tumors; in the control groups, pituitary adenomas and mammary tumors. A few rats in both the exposed and control

groups were found to have zymbal gland tumors or brain tumors. Although four angiosarcomas occurred in mesenteric lymph nodes and one in the mediastinum in exposed rats, these were not considered to be induced by vinyl chloride since one nonhepatic angiosarcoma (subcutaneous) was also seen in control rats in this experiment and have been seen in control rats in other experiments in a low incidence ($< 1\%$). However, one early angiosarcoma of the spleen and two metastatic angiosarcomas in the lungs (one from a primary in the mesentery) were seen in exposed rats. These were considered to be induced by vinyl chloride and are included in the angiosarcoma statistics.

Since the first liver angiosarcoma did not occur until the 16th week of the study, only those animals alive at that time were considered to be at risk for the development of angiosarcomas. Those are the animals used in the incidence statistics.

A total of 28 out of 370 males and 50 out of 387 females in all exposed groups combined were found to have angiosarcomas. Only one angiosarcoma was seen in all the controls (group 5, male) and it was subcutaneous. The greatest percentage of angiosarcomas were found by far in the nonscheduled sacrifice groups. The exposure times, mean survival times, number of animals at risk, incidence of angiosarcomas and the week that the first angiosarcoma was diagnosed for each age group of males in the nonscheduled sacrifice groups are tabulated (Table 3). Although no angiosarcomas were observed in the two youngest age groups, they were seen in 3/45 (6.7%) of the rats in group 6 and 13/55 (24%) of the rats in group 8. The first angiosarcoma occurred 7 weeks earlier in group 8 than in group 6. Histologically, the angiosarcomas were highly anaplastic and most of them had metastasized.

Similar data concerning the female rats in the nonscheduled sacrifice groups are also tabulated (Table 3). Angiosarcomas were seen in all groups with increasing frequency from group 2 (youngest)

Table 3. Angiosarcomas in exposed male and female rats in nonscheduled sacrifice groups.

Group	Sex	Mean exposure, weeks	Mean survival, weeks	Rats at risk	Angiosarcoma incidence	Incidence of metastases	First appearance of angiosarcomas, weeks
2	M	24.5	30.6	37	0/37	—	—
2	F	24.6	37.5	38	2/38 (5.3%)	2/2	30
4	M	24.4	31.6	44	0/44	—	—
4	F	24.7	33.8	47	7/47 (15%)	5/7	28
6	M	25.0	30.5	45	3/45 (6.7%)	3/3	28
6	F	24.0	30.0	49	23/49 (47%)	10/23	20
8	M	24.5	28.9	55	13/55 (24%)	11/13	21
8	F	23.9	30.2	54	11/54 (20%)	6/11	16

to group 6. The incidence in group 8, however, is lower than in group 6 and closer to that in group 4. The first angiosarcoma, however, was seen in group 8, the oldest group.

In comparing the males and females, it can be seen in Table 3 that the first angiosarcoma appeared earlier in the females in each group and that the incidence was higher in females for each age group, with the exception of group 8, the oldest age category. Since the mean survival times and exposure durations for males and females in groups 4 and 6 were similar, the differences in incidence can be attributed to sex.

The incidences of angiosarcomas in the interim sacrifice, nonscheduled sacrifice and combined groups appear in Table 4. The females sacrificed at 6 and 9 months had a much lower incidence of angiosarcomas than those in the nonscheduled sacrifice groups, whereas, the difference in incidence between similar groups of males was not as great.

The incidences of angiosarcomas in the different age groups were compared using Fisher's exact test. Because of multiple comparisons, the α -level for each individual comparison needed to be ≤ 0.01 for the incidences to be significantly different with an overall $\alpha = 0.05$. In the nonscheduled sacrifice groups, the incidence of angiosarcomas in group 8 males was significantly different from those incidences in group 4 and group 2 males; the incidence of angiosarcomas in group 6 females was significantly different from those in group 4, group 2 and group 8 females. The same comparisons between the same groups in the combined nonscheduled and interim sacrifice groups were also found to be significant.

The effect of age on angiosarcoma incidence in males was examined using the method of weighted least squares regression. A quadratic equation was shown to be a good model for predicting angiosarcoma incidence as a function of age in weeks. The percent of variability in incidence rates (R^2) explained by this model was: $R^2 = 99.96\%$ for nonscheduled sacrifice groups and $R^2 = 99.85\%$ for combined

nonscheduled and interim sacrifice groups (Fig. 1). Both of these models were statistically significant ($p = 0.02$ and $p = 0.001$, respectively).

The fact that most of the angiosarcomas in the females occurred in the animals that died spontaneously suggested that their deaths might be directly related to the angiosarcomas. A comparison of mean survival times, however, revealed that animals with angiosarcomas survived as long and frequently longer than other animals in their respective groups (Table 5). At 4-week intervals, the accumulative incidence of angiosarcomas was tabulated (Table 6) for exposed females. Although the incidence of angiosarcomas at all intervals in groups 2 and 6 is about the same, in group 8 the incidence decreases with time, and in group 4 it increases with time.

Although a few brain tumors, mammary adenocarcinomas and zymbal gland tumors occurred in exposed animals, their incidences did not appear to be significantly different from those of the controls.

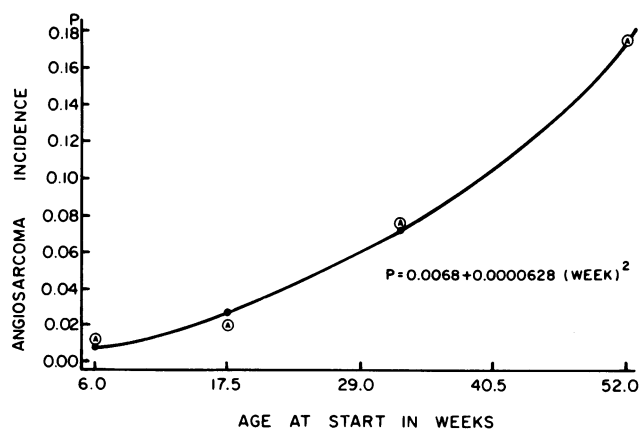


FIGURE 1. Angiosarcoma incidence: males (combined deaths, interim sacrifices and final sacrifice). Curve fitted by regression methods showing a significant quadratic fit. $R^2 = 99.85\%$.

Table 4. Incidence of angiosarcomas in exposed rats, nonscheduled (NS), interim sacrifice (IS) and combined groups.

Group	Sex	NS	IS	Combined NS + IS
2	M	0/37	1/46 (2.2%)	1/83 (1.2%)
2	F	2/38 (5.3%)	0/50	2/88 (2.3%)
4	M	0/44	2/47 (4.3%)	2/91 (2.2%)
4	F	7/47 (15%)	0/50	7/97 (7.2%)
6	M	3/45 (6.7%)	4/49 (8.2%)	7/94 (7.4%)
6	F	23/49 (47%)	4/49 (8.2%)	27/98 (28%)
8	M	13/55 (24%)	5/47 (11%)	18/102 (18%)
8	F	11/54 (20%)	3/50 (6%)	14/104 (13%)

Table 5. Mean survival of exposed rats in nonscheduled sacrifice groups.

Group	Mean survival, weeks			
	Males		Females	
	All rats	Rats with angiosarcomas	All rats	Rats with angiosarcomas
2	30.6	—	37.5	36.5
4	31.6	—	33.8	38.9
6	30.5	35.3	30.0	32.0
8	28.9	31.2	30.2	27.3

Table 6. Accumulative angiosarcoma incidence in exposed females, nonscheduled sacrifice group.

Interval, weeks	Angiosarcoma incidence			
	Group 2	Group 4	Group 6	Group 8
16-19	0/0	0/1	0/2	2/5 (40%)
20-23	0/1	0/5	3/7 (43%)	3/9 (33%)
24-27 ^a	0/6	0/14	8/23 (35%)	6/22 (27%)
28-31	1/13 (7.7%)	1/22 (4.5%)	12/31 (39%)	8/34 (24%)
32-35	1/14 (7.1%)	2/28 (7.1%)	14/37 (38%)	10/40 (25%)
36-39	1/15 (6.7%)	3/29 (10%)	18/41 (44%)	11/46 (24%)
40-43	2/38 (5.3%)	7/47 (15%)	23/49 (47%)	11/54 (20%)

^aExposures terminated.

Discussion

The results clearly indicate that the incidence of angiosarcomas is higher and these tumors occur earlier in older rats of both sexes. In addition, the incidence of angiosarcomas is generally higher and these tumors occur earlier in female rats than in male rats. Therefore, it can be concluded that older rats are more susceptible to the angiosarcoma-inducing effect of vinyl chloride than are young adult rats and that female rats are more susceptible than males.

The authors have been unable to find comparable studies in the scientific literature. However, Maltoni (1) reported in a summary article of his research with vinyl chloride that exposure duration was an important factor in determining angiosarcoma incidence. In that article, he reported that rats exposed to 10,000 ppm vinyl chloride, 4 hr/day, 5 days/week for 17 weeks did not develop liver angiosarcomas within a 155-week period, whereas, 13/60 (22%) of rats exposed to 6,000 ppm vinyl chloride 4 hr/day, 5 days/week for 52 weeks (and held for a 155-week period) developed liver angiosarcomas. In the authors' opinion, the difference in total dose between the two groups was not sufficient to account for the large difference in the angiosarcoma incidences, and that the major factor, therefore, was probably the difference in ages while they were being exposed.

There is suggestive evidence in the literature that older adult humans might be more susceptible than young adults to the carcinogenic effects of Thorotrast. Curry et al. (2) stated in their article that "although the latent period between thorium injection and liver malignancy has varied from 3 years to 35 years, all of these 123 cases occurred in

patients between 49 and 55 years of age." There is suggestive evidence that older beryllium workers are more susceptible to the carcinogenic effects of beryllium. The data of Mancuso's study (3) of beryllium extraction workers show an extremely high risk for lung cancer in workers between the ages of 38 and 65 who were exposed for relatively short periods of time. Studies specifically designed to test this theory in humans, as well as in animals, with other compounds are needed.

If these findings can be reproduced in animals with a wide variety of classes of compounds, then it would be justifiable to modify chronic bioassay experiments by utilizing older animals at the beginning of the studies and shortening the durations of the experiments by 6-12 months. In many cases, this should result in decreasing costs by 20-30% and, thereby, permit a greater number of chemicals to be tested.

The observation in this study that the accumulative incidence of angiosarcomas in the 6-week-old group of rats did not increase with time after discontinuation of the exposures suggests that the carcinogen is metabolized and inhibited or excreted before most of the animals became susceptible. Whether or not these animals would have exhibited a higher incidence of angiosarcoma at some later time is not known. However, as mentioned above, Maltoni (1) was unable to induce liver angiosarcomas in young adult rats exposed to 10,000 ppm vinyl chloride 4 hr/day, 5 days/week for 17 weeks and held for a lifetime. The total dose in his experiment was 10,000 ppm $\times$ 340 hr (3,400,000 ppm-hr). The total dose in our experiment was 948 ppm $\times$ 858 hr (813,384 ppm-hr). Therefore, it is unlikely that the youngest groups in our experiment would have developed a higher incidence of angiosarcomas if they were held for a lifetime.

The results of this study also suggest that older people should not be preferentially placed in carcinogenic working environments.

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