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Toxicity of Intraperitoneal Injections of 7,12-Dimethylbenz[a]anthracene in Inbred Mice[1]

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SUMMARY

The sensitivity of A/HeJ, AKR/J, C3H/HeJ, C57BL/6J, C58/J, and DBA/2J male mice to single i.p. injections of 7,12-dimethylbenz[a]anthracene (DMBA) was investigated. The drug was administered in a single dose of 0.75 to 3 mg per mouse and animals were sacrificed 5–20 days after injection. The AKR mice were least affected by DMBA. Acute peritoneal inflammation accompanied by accumulation of an exudate was pronounced in the C57BL/6, A/He, C58, C3H/He mice and only slight in DBA/2 and AKR mice. Depression of the lymphocyte count in the peripheral blood and decrease of the weight of spleen and thymus were also more pronounced in strains with increased mortality rates and severe peritoneal inflammation.

Toxicity remained the same whether sesame oil, dimethyl sulfoxide, or hexadecane were the solvents, but decreased when DMBA was suspended in physiologic saline containing Tween 80.

Male and female AKR and C57BL/6 mice responded the same to treatment.

INTRODUCTION

In testing drugs for toxicity we have noticed that i.p. administrations of DMBA were more toxic to C57BL/6 than to AKR mice.

Studies of the influence of strain specificity on the induction of skin tumors by chemicals revealed that C57BL mice showed more severe toxic reactions than other mouse strains. Mider and Morton (8) observed that C57BL mice were ill after 12 biweekly paintings of an 0.5% solution of 3-methylcholanthrene in benzene. Andervont and Edgcomb (1) applied weekly an 0.25% solution of 3-methylcholanthrene in benzene to the skin of A, BALB/c, C57BL, C3H, DBA/2, I, and RIII mice. The C57BL mice were the only strain to exhibit any deleterious effects. Gilman (5) made similar observations in regard to the high sensitivity of this strain to the toxic effects of cutting oils, tobacco tars, and croton oil. Shubik and Sicé (12) considered the C57BL mouse unsatisfactory for skin painting studies, since their skin often ulcerates.

In view of these findings and because of the well-known differences in spontaneous tumor incidence between mouse strains (6) it was deemed important to study the toxic effects of DMBA administered i.p. in various inbred mouse strains.

MATERIALS AND METHODS

Mice, obtained from the Jackson Laboratory, were 5–8 weeks old at the beginning of the experiment.

DMBA was obtained from Eastman Organic Chemicals Department, Rochester, N. Y. It was 99.5+% pure as determined by gas chromatography by F. M. Archibald and was also checked by ultraviolet absorption spectroscopy by D. Hoffmann of this institute. Taking as reference Sandin and Fieser, J. Am. Chem. Soc., *62:* 3098, 1940, the log E at 364 mμ is 3.94. The average log E of 3 runs was 3.93. The results, therefore, are within an experimental error of 3%. The paper chromatogram showed trace impurities when exposed to a short wave ultraviolet lamp (254 mμ). Sesame oil (Benne), U.S.P., *n*-hexadecane, and dimethyl sulfoxide were purchased from Fisher Scientific Company, Fair Lawn, N. J.

DMBA was administered i.p. as a single dose of 0.75 mg and 1.5 mg/mouse dissolved in 0.1 ml, or of 3 mg/mouse dissolved in 0.2 ml of sesame oil. To study the role of the vehicle, dimethyl sulfoxide, hexadecane, and physiologic saline containing 1 drop of Tween 80 per 15 ml were used.

Five, 10, and 20 days after injection groups of mice were killed and autopsies were performed. The body, spleen, and thymus weights were recorded. The differences in body weights of mice with and without peritoneal exudate represented the ascites values.

Samples for blood counts were taken by orbital bleeding. For the differential counts blood smears were made and stained with Wright's stain. Monocytes and intermediate forms between monocytes and lymphocytes were pooled and included in the lymphocytic count.

For the determination of bone marrow cellularity mice were killed by cervical dislocation and both femurs removed. The marrow cavities were opened at both ends with a drill and the bone marrow was flushed out and diluted with 2 ml of 3% solution of acetic acid. The nucleated cells in this suspension were counted in the hemocytometer.

RESULTS

Toxic effects following a single i.p. injection of DMBA in male mice of various mouse strains are presented in Tables 1 and 2. There were no deaths by Day 5 in any group. After 5 days the mortality of mice of different strains varied. DMBA caused less

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TABLE 1

Effect of DMBA[a] in AKR and DBA/2 Male Mice

Strain	Day after injection	DMBA[b] (mg/mouse)	Mortality	Av. initial body wt. (gm)	Body wt. change (gm)	Av. spleen wt. (mg)	Av. thymus wt. (mg)	Av. ascites (gm)
AKR	10	Control	0/20	21.4 ± 1.0	+2.6	82 ± 12	94 ± 15	0
	5	0.75	0/20	21.6 ± 2.0	−0.7	52 ± 10	96 ± 13	0
		1.5	0/20	22.5 ± 2.3	−1.8	41 ± 3	91 ± 11	0
		3.0	0/20	21.5 ± 2.6	−1.5	37 ± 6	84 ± 8	0
	10	0.75	0/20	19.8 ± 1.9	+1.3	63 ± 11	87 ± 15	0
		1.5	0/20	21.0 ± 2.2	−0.4	49 ± 13	83 ± 16	0
		3.0	1/20	21.2 ± 2.1	−0.3	31 ± 9	67 ± 20	0.9 ± 0.8
	20	0.75	0/20	21.5 ± 1.5	+1.8	72 ± 12	80 ± 12	0
		1.5	2/20	21.9 ± 1.5	−0.6	52 ± 10	53 ± 11	0
		3.0	5/20	21.1 ± 1.6	+1.2	39 ± 21	63 ± 10	1.9 ± 1.0
DBA/2	10	Control	0/10	20.2 ± 2.8	+0.4	90 ± 16	77 ± 17	0
	5	0.75	0/10	21.1 ± 1.5	−0.6	56 ± 7	45 ± 10	0
		1.5	0/10	20.9 ± 1.7	−1.1	45 ± 6	38 ± 9	0
		3.0	0/10	19.4 ± 2.1	−1.8	37 ± 5	35 ± 10	0
	10	0.75	1/10	19.9 ± 1.2	−0.8	42 ± 7	44 ± 14	0
		1.5	1/10	20.0 ± 0.7	−2.4	28 ± 8	40 ± 10	0.6 ± 0.4
		3.0	5/10	20.2 ± 1.8	−0.4	21 ± 5	26 ± 4	1.3 ± 0.7
	20	0.75	1/10	19.9 ± 0.7	−1.4	73 ± 19	37 ± 14	0
		1.5	5/10	19.7 ± 0.9	−2.8	52 ± 25	19 ± 3	0.5 ± 0.1
		3.0	10/10	22.4 ± 1.4				

[a] DMBA, 7,12-dimethylbenz[a]anthracene.

[b] DMBA dissolved in 0.1 or 0.2 ml of sesame oil was administered i.p. Controls received 0.2 ml of sesame oil i.p.

deaths in the AKR than in any other strain. Only 5 of 20 mice treated with 3 mg/mouse, 2 of 20 mice receiving 1.5 mg/mouse, and none of the mice treated with 0.75 mg/mouse died by Day 20.

The DBA/2 mice (Table 1) also showed a low mortality. Following DMBA treatment with 0.75, 1.5, and 3 mg/mouse, 1, 5, and 10 mice, respectively, died by Day 20.

In contrast to AKR and DBA/2 mice the mortality was high and similar in the C57BL/6, C58, C3H, and A/He mice (Table 2). All of the C57BL/6 mice treated with 3 mg or 1.5 mg DMBA per mouse, and 17 of the 20 mice treated with 0.75 mg were dead by Day 20.

AKR and DBA/2 mice showed less loss in body weight than the other strains. Following all 3 dose levels of DMBA the AKR and DBA/2 mice (Table 1) showed less than 2 gm weight loss by Day 5 whereas the C57BL/6, A/He, C58, and C3H/He mice (Table 2) lost more than 2 gm after 1.5 and 3.0 mg of DMBA. The body weight losses in general were dose related.

At autopsy the peritoneal cavities showed acute inflammation of the peritoneum accompanied by the accumulation of fluid. On Day 5 the exudate was cloudy or milky and by Days 10 and 20 it was milky and sometimes hemorrhagic. By Day 10 the exudate was often fibrinous and adhesions started to form between the organs. The exudate in A/He mice was less viscous than in C57BL/6, C58, and C3H mice. The i.p. fluid taken from C57BL/6 mice on Day 5 contained polymorphonuclear leukocytes, lymphocytes, and a few monocytes. From Day 10 on only a few cell shadows and protein particles could be seen. Small amounts of fluid in the pleural cavities were usually concomitant with severe cases of fluid accumulation in the peritoneal cavity. Only slight amounts of peritoneal exudate were found in the AKR mice treated with 3 mg DMBA and in the DBA/2 mice treated with 1.5 or 3 mg/mouse, whereas all treated groups of the other strains contained from 0.8 to 3.4 ml peritoneal exudate.

In the control mice the thymus weight was the greatest in the AKR mice. The spleen weights were in the same range in all the strains. Treatment with DMBA affected the weight of the thymus more than the spleen. A close correlation existed between mortality and the reduction in weight of thymus and spleen. Spleens of the mice which died from DMBA intoxication weighed less than 20 mg and the thymuses were either invisible or so small that they could not be weighed accurately. A marked decrease in spleen and thymus weight occurred in the strains C57BL/6, A/He, C3H, and C58 (Table 2), a moderate decrease in the DBA/2 mice and the least in AKR mice (Table 1). Following DMBA doses of 0.75, 1.5, and 3.0 mg/mouse the spleen weights for AKR mice at Day 20 were 72, 52, and 39 mg, respectively. In contrast, all the C57BL/6 mice treated with 1.5 and 3.0 mg/mouse were dead by Day 20 and the average spleen weight of the 3 surviving mice was 30 mg. Even more striking was the difference in the weights of the thymus glands of these 2 strains. There was only a moderate decrease following all 3 doses in the AKR mice, whereas in the C57BL/6 mice the thymus glands were not noticeable after a dose of 0.75 mg/mouse.

Table 3 shows the effects induced by a single injection of 1.5 mg/mouse of DMBA on white blood cell counts and bone marrow

TABLE 2

Effect of DMBA[a] in C57BL/6, C58, C3H/He, and A/He Male Mice

Strain	Day after injection	DMBA[b] (mg/mouse)	Mortality	Av. initial body wt. (gm)	Body wt. change (gm)	Av. spleen wt. (mg)	Av. thymus wt. (mg)	Av. ascites (gm)
C57BL/6	10	Control	0/20	21.4 ± 0.9	+0.7	99 ± 18	58 ± 17	0
	5	0.75	0/20	20.6 ± 0.6	−1.5	41 ± 11	44 ± 11	1.1 ± 0.9
		1.5	0/20	20.6 ± 1.3	−2.6	23 ± 5	23 ± 7	1.7 ± 0.6
		3.0	0/20	21.2 ± 2.1	−3.2	22 ± 11	<15	1.9 ± 0.6
	10	0.75	0/20	20.8 ± 1.1	−0.4	32 ± 13	22 ± 7	2.5 ± 1.1
		1.5	4/20	21.0 ± 4.7	−3.4	16 ± 4	<15	1.9 ± 0.7
		3.0	19/20	21.0 ± 2.2	−4.0	27	28	3.0
	20	0.75	17/20	21.5 ± 1.6	−2.4	30 ± 6	<15	3.4 ± 1.4
		1.5	20/20	21.3 ± 1.4				
		3.0	20/20	21.2 ± 0.8				
C58	10	Control	0/10	19.3 ± 2.5	+1.3	127 ± 30	62 ± 12	0
	5	0.75	0/10	19.2 ± 0.5	−1.4	60 ± 22	37 ± 12	0.9 ± 0.6
		1.5	0/10	19.9 ± 0.8	−3.3	27 ± 5	21 ± 5	1.5 ± 0.5
		3.0	0/9	19.8 ± 2.2	−2.8	22 ± 8	18 ± 8	1.2 ± 0.5
	10	0.75	2/10	19.2 ± 0.8	−2.6	41 ± 31	37 ± 13	1.6 ± 0.6
		1.5	7/10	19.7 ± 1.1	−5.8	12 ± 3	<15	1.2 ± 0.3
		3.0	8/10	19.2 ± 2.2	−0.6	19, 25	<15	3.8, 2.5
	20	0.75	2/10	19.3 ± 0.9	−0.4	35 ± 14	26 ± 21	2.6 ± 0.9
		1.5	9/10	19.4 ± 0.6	−6.0	14	<15	1.8
		3.0	10/10	20.0 ± 2.5				
C3H/He	10	Control	0/10	19.9 ± 0.7	−1.3	102 ± 36	35 ± 13	0
	5	0.75	0/10	19.9 ± 0.6	−3.1	34 ± 7	27 ± 9	0.8 ± 0.4
		1.5	0/10	20.3 ± 1.2	−3.2	22 ± 5	25 ± 5	1.1 ± 0.4
		3.0	0/10	21.0 ± 3.4	−4.2	16 ± 3	17 ± 8	1.2 ± 0.4
	10	0.75	1/10	20.1 ± 1.7	−2.2	38 ± 17	28 ± 15	1.6 ± 1.4
		1.5	5/10	20.3 ± 1.1	−0.9	27 ± 10	28 ± 8	1.8 ± 1.8
		3.0	10/10	20.4 ± 2.1				
	20	0.75	7/10	20.0 ± 1.9	−0.9	54 ± 31	22 ± 9	1.4 ± 1.3
		1.5	8/10	20.3 ± 0.6	−6.5	19, 18	18, 14	1.4, 0.8
		3.0	10/10	23.8 ± 1.4				
A/He	10	Control	0/10	19.4 ± 4.2	+2.2	111 ± 41	53 ± 12	0
	5	0.75	0/10	20.3 ± 2.5	−1.7	35 ± 7	27 ± 8	1.0 ± 0.9
		1.5	0/10	20.3 ± 2.4	−3.3	27 ± 7	<15	1.0 ± 0.8
		3.0	0/10	19.5 ± 1.8	−3.7	20 ± 8	<15	1.2 ± 0.6
	10	0.75	0/9	20.6 ± 2.8	−2.5	31 ± 11	18 ± 8	1.9 ± 0.5
		1.5	7/10	20.3 ± 2.2	−8.2	18 ± 3	<15	1.1 ± 0.8
		3.0	9/10	20.7 ± 0.9	−8.0	14	<15	0.7
	20	0.75	8/12	19.4 ± 4.1	−5.4	30 ± 18	<15	1.6 ± 1.2
		1.5	10/10	20.6 ± 0.8				
		3.0	10/10	20.5 ± 0.7				

[a] DMBA, 7,12-dimethylbenz[a]anthracene.

[b] DMBA dissolved in 0.1 or 0.2 ml of sesame oil was administered i.p. Controls received 0.2 ml of sesame oil i.p.

cellularity in AKR and C57BL/6 mice. The essential difference was a pronounced depression of the peripheral lymphocytes in the C57BL/6 mice. Five days after injection the lymphocyte count was 21% of the normal count and it decreased to 10% by Day 10. Concomitant with the fall of the lymphocytes in the C57BL/6 mice the bone marrow cellularity sharply decreased.

Male and female AKR and C57BL/6 mice responded the same to DMBA (Table 4) in respect to spleen weight, thymus weight, and the formation of ascites. The females weighed approximately 19 gm each, i.e., 1–2 gm less than the males, at the time of injection. Therefore it was not surprising that the same amount of DMBA was slightly more effective in the females.

Sesame oil has been used as standard solvent for DMBA in our studies. For comparison, the effects of dimethyl sulfoxide, hexadecane, and physiologic saline containing 1 drop of Tween 80 per 15 ml upon the irritating ability of DMBA are shown in Table 5. The data obtained with dimethyl sulfoxide and hexadecane as solvents are comparable with those obtained with sesame oil. With saline and Tween the toxicity was slightly reduced.

TABLE 3

Effect of DMBA[a, b] on White Blood Cells and Bone Marrow Cellularity

Strain	Day after injection	Lymphocytes		Polymorphs		Marrow count (× 10^6 cells)
		Mean (10^3/cu mm)	% Normal count	Mean (10^3/cu mm)	% Normal count	
AKR male	Control	4.8 ± 1.5	100	3.5 ± 1.7	100	35.1 ± 3.4
	5	3.7 ± 0.9	77	2.3 ± 2.0	66	28, 32, 32
	10	5.2 ± 1.2	108	1.9 ± 1.3	54	30, 30, 29
C57BL/6 male	Control	6.7 ± 1.6	100	1.9 ± 0.6	100	48.0 ± 5.9
	5	1.4 ± 0.5	21	1.4 ± 0.4	73	31, 36, 33
	10	0.7 ± 0.6	10	3.1 ± 1.3	163	21, 22, 24

[a] DMBA, 7,12-dimethylbenz[a]anthracene.

[b] 1.5 mg in 0.1 ml of sesame oil i.p. per mouse. The marrow counts on Days 5 and 10 were done on 3 mice. The other groups consisted of 10 mice.

TABLE 4

Effect of DMBA[a] in AKR and C57BL/6 Female Mice

Strain	Day after injection	DMBA[b] (mg/mouse)	Mortality	Av. initial body wt. (gm)	Body wt. change (gm)	Av. spleen wt. (mg)	Av. thymus wt. (mg)	Av. ascites (gm)
AKR female	10	Control	0/10	19.7 ± 2.8	+0.2	86 ± 16	91 ± 25	0
	5	1.5	0/10	19.7 ± 0.8	−1.9	42 ± 7	89 ± 16	0
	10	1.5	0/10	19.8 ± 0.8	−1.1	41 ± 5	106 ± 23	0
	20	1.5	0/10	19.9 ± 0.6	−5.7	48 ± 10	37 ± 13	0
C57BL/6 female	10	Control	0/10	19.0 ± 0.4	−0.4	89 ± 13	70 ± 4	0
	5	1.5	0/10	19.3 ± 1.8	−1.2	20 ± 4	26 ± 8	1.6 ± 0.3
	10	1.5	8/10	19.6 ± 1.4	−0.2	10, 8	<15	1.5, 1.9
	20	1.5	10/10	19.7 ± 1.3				

[a] DMBA, 7,12-dimethylbenz[a]anthracene.

[b] DMBA dissolved in 0.1 ml of sesame oil was administered i.p. Controls received 0.2 ml of sesame oil.

TABLE 5

Effect of Various Solvents on the Toxicity of Intraperitoneally Applied DMBA[a] (Autopsy 20 Days after Injection)

Strain	Vehicle	DMBA (mg/20 gm body wt.)	Mortality	Av. initial body wt. (gm)	Body wt. change (gm)	Av. spleen wt. (mg)	Av. thymus wt. (mg)	Av. ascites (gm)
AKR male	DMSO	0[b]	0/5	21.5 ± 0.6	+2.7	69 ± 6	98 ± 14	0
		1.5[c]	3/10	21.4 ± 1.1	−5.5	35 ± 10	44 ± 19	0
	n-Hexadecane	0	0/5	23.3 ± 0.6	+1.4	95 ± 6	73 ± 12	0
		1.5[c]	1/10	22.2 ± 1.4	−3.1	61 ± 13	42 ± 15	0.5 ± 0.2
	Saline (+ Tween 80)	0	0/5	24.1 ± 0.9	0	70 ± 10	49 ± 3	0
		1.5[d]	0/10	22.0 ± 2.0	−3.3	37 ± 11	43 ± 20	0
C57BL/6 male	DMSO	0	0/5	21.3 ± 1.0	+2.0	90 ± 6	65 ± 4	0
		1.5	10/10	21.5 ± 1.1				
	n-Hexadecane	0	0/5	23.6 ± 0.6	−0.1	154 ± 5	40 ± 7	0
		1.5	8/10	22.7 ± 0.7	+0.7	25, 46	22, 38	5.5, 4.9
	Saline (+ Tween 80)	0	0/5	23.4 ± 0.5	−0.3	100 ± 18	57 ± 21	0
		1.5	4/10	22.9 ± 1.3	+2.0	35 ± 24	28 ± 19	3.0 ± 1.9

[a] DMBA, 7,12-dimethylbenz[a]anthracene; DMSO, dimethyl sulfoxide.

[b] Controls received 0.1 ml of the respective vehicle.

[c] 1.5 mg dissolved in 0.1 ml.

[d] 1.5 mg/0.1 ml of saline. One drop of Tween 80 was added to the total volume of 15 ml.

DISCUSSION

The responses of several commonly used inbred mouse strains to DMBA inoculated i.p. indicate that the strains fall into 2 major groups. The C57BL/6, C58, A/He, C3H/He mice (Table 2) were much affected by DMBA whereas the DBA/2 and especially the AKR mice (Table 1) showed little response.

The pathologic picture was qualitatively similar in all the strains but quantitatively they differed greatly. The local toxicity was expressed by symptoms of peritoneal inflammation, whereas systemic effects were mainly reflected by changes in spleen weight, thymus weight, and blood count.

Basically the pathologic findings were the same as those observed by Shubik and Della Porta (11) in Swiss mice treated with large i.p. doses of DMBA. All 5 mice treated with 25 mg DMBA/mouse in olive oil died on the 5th day after injection. Characteristic changes occurred in the spleen, lymph nodes, bone marrow, testes, intestinal mucosa, and blood.

The special sensitivity of the skin of C57BL mice to irritants has long been known (1, 5, 8, 12). The present study shows that the C57BL/6 mouse is equally sensitive to i.p. application of DMBA. Shimkin (9) reported the development of skin ulceration in A mice following s.c. administration of methylcholanthrene. In the present study A mice showed a high degree of sensitivity to DMBA.

Stamer (13) and Shubik and Della Porta (11) found after parenteral application of large doses of DMBA in Swiss mice a degree of leukopenia consistent with the results obtained in the present study in C57BL/6 mice. The lower sensitivity of the AKR mice was reflected in smaller pathologic changes of the blood and marrow counts.

No correlation exists between the incidence of spontaneous tumors in a mouse strain (6, 10) and i.p. sensitivity to DMBA as shown herein. At present it is not known whether the differential sensitivity to DMBA is because of a difference in hormonal stimulation or of a difference of the target cells. The same question remains unanswered for most genetic differences in spontaneous tumor incidence.

The incidence of tumors induced with minimal effective doses of cancerigenic hydrocarbons has been found to differ in male and female mice (7), but not if larger doses were used (4, 14). The authors observed (unpublished report) that a single s.c. injection of 15 μg/mouse of DMBA in 0.2 ml of sesame oil caused formation of tumors in all 30 injected C57BL/6 mice. In view of these results the dose of 1.5 mg used in the present study was so large that no sex difference was apparent.

Objections to the use of natural oils (sesame oil, etc.) as vehicles for cancerigenic hydrocarbons because of their variable and complex composition have been repeatedly expressed in the literature (2, 3, 7, 9). Under the conditions of the present study sesame oil was as effective a vehicle as the chemically defined solvents, hexadecane and dimethyl sulfoxide. Saline with Tween proved to be slightly less effective, probably because DMBA is a suspension in this vehicle, whereas it is soluble in the other vehicles.

Differential toxicity studies in various inbred mouse strains appear to offer an approach to better knowledge of physiologic factors in cancerigenesis.

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