1,2-di-*n*-butylhydrazine dihydrochloride carcinogenesis in mice

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Summary. A solution of 0.0625% 1,2-di-*n*-butylhydrazine dihydrochloride (1,2-DBH) was given continuously in the drinking water of 6-week-old randomly bred albino Swiss mice for the remainder of their lives. The treatment gave rise to tumors of the lungs, lymphoreticular tissue and kidneys.

One of the aims in studying substituted hydrazines is to investigate the effect of chemical structure on tumor development at specific organ sites. For this reason, we have been conducting a series of investigations in mice in the hope of determining the relative carcinogenic potency of mono-, 1,1-, and 1,2-disubstituted hydrazines. To date, studies with methyl-[1], 1,2-dimethyl-[2], 1.1-dimethyl-[3], formyl-[4], 1,2-diformyl-[5] and *n* butylhydrazine[6] have been completed in the Swiss mouse. All of these chemicals were administered in the drinking water for life at the maximum tolerated dose levels. The present work is a direct continuation of our past efforts and demonstrates the carcinogenicity of 1,2-di-*n*-butylhydrazine dihydrochloride given under conditions identical to those of our previous studies to Swiss mice.

Materials and methods. Swiss albino mice from the colony randomly bred by us since 1951 were used. They were housed in plastic cages with granular cellulose bedding, were separated according to sex in groups of 5, and were given Wayne Lab-Blox diet in regular pellets (Allied Mills, Inc., Chicago, Ill.) and tap water or the chemical solution ad libitum as described below. The chemical used was 1,2-di-*n*-butylhydrazine dihydrochloride (1,2-DBH), CH_3-CH_2-CH_2-CH_2-NH-NH-CH_2-CH_2-CH_2-$CH_3 \cdot 2HCl$, mol.wt: 217.18; m.p.: > 280 °C, purity > 98%, and was synthesized in this laboratory as previously described[7] and isolated as the dihydrochloride salt (m.p.: 154–156 °C). Toxicity studies were carried out with 1,2-DBH prior to the chronic experiments. 5 dose levels 0.5, 0.25, 0.125, 0.0625 and 0.03125% were administered in the drinking water for 35 days to Swiss mice. By taking into account 4 parameters the survival rates, body weights, chemical consumption figures and histological changes, the 0.0625% dose level was found to be suitable for the lifelong treatment. This toxicity technique was developed in this laboratory[8]. The solutions were prepared 3 times weekly and the total consumption of water containing 1,2-DBH was measured at the same intervals during treatment period. The solutions were contained in brown bottles because of the possible light sensitivity of the chemical. After standing 48 h at room temperature in a dark brown bottle, an aqueous solution of 0.0625% 1,2-DBH was neutralized and extracted into methylene chloride and analyzed by gas chromatography and found to contain greater than 99% of the unchanged compound (after adjustment for extraction efficiency). Upon standing in methylene chloride the extract exhibited a UV peak at 363 nm which increased with time. This peak is indicative of azo compounds[9]. Analysis of the aqueous solution of the dihydrochloride exhibited no UV absorption after standing however. The chronic experimental groups were the following: Group 1: 1,2-DBH was dissolved in the drinking water as a 0.0625% solution and was given for the lifespan of 50 female and 50 male mice that were 6 weeks (44 days) old at the beginning of the experiment. The average daily consumption of water containing 1,2-DBH per animal was 5.8 ml for the females and 9.03 ml for the males. Therefore, the average daily intake of 1.2-DBH was 3.64 mg for a female and 5.64 mg for a male.

Group 2: As an untreated control, 100 female and 100 male mice were kept and observed from weaning time (6 weeks of age). The experimental and control animals were carefully checked and weighed at weekly intervals, and the gross pathological changes were recorded. The animals either were allowed to die or were killed with ether when found in poor condition. Complete necropsies were performed on all animals. All organs were examined macroscopically and were fixed in 10% buffered formalin. Histological studies were done on the liver, spleen, kidney, bladder, thyroid, heart, pancreas, testes, brain, nasal turbinale, and at least 4 lobes of the lungs of each mouse, as well as on those organs showing gross pathological changes. Sections from these tissues were stained routinely with hematoxylin and eosin.

Results. The survival rates after weaning are described in table 1. As can be seen from the data, the treatment substantially shortened the survival in both sexes, when compared to that in controls. The number, percentages of mice with tumors, and their ages at death (latent periods) are summarized in table 2. The 3 statistically significant neoplasms are described in detail as follows: Lung tumors. Of the treated females, 36 (72%) developed 186 tumors of this organ. Of these, 15 mice had 37 adenomas, 1 mouse had 4 adenocarcinomas and 20 mice developed 100 adenomas and 45 adenocarcinomas. In the treated males, 38 (76%) developed 208 lung neoplasms. Of these, 8 mice had 23 adenomas, 1 mouse developed 3 adenocarcinomas and 29 mice developed 115 adenomas and 67 adenocarcinomas. Grossly and histologically these lung lesions were similar to those described earlier in this laboratory[10,11]. Malignant lymphomas. Of the treated females, 21 (42%) developed lymphoreticular tissue neoplasms. Of these, 19 were classified as lymphocytic type and the remaining 2 as histocytic type. In the treated males, 6 (12%) developed malignant lymphomas, lymphocytic type. Macroscopically and histologically these neoplasms were similar to those described earlier by us[12]. Kidney tumors. Of the treated males, 4 (8%) developed tumors of this organ. Of these, 3 were classified

Table 1. Treatment and survival rates in 1,2-di-*n*-butylhydrazine dihydrochloride (1,2-DBH) treated and control Swiss mice

Group	Treatment	Initial No. and sex of mice	No. of survivors (age in weeks) 10	20	30	40	50	60	70	80	90	100	110	120	130	140	150
1	0.0625% 1,2-DBH in drinking water daily for life	50♀	50	50	48	43	39	36	26	17	5	–					
		50♂	50	48	46	44	42	38	26	9	1	–					
2	Untreated control	100♀	100	99	98	92	83	77	70	55	45	30	16	6	1	1	–
		100♂	100	100	100	96	91	81	63	44	28	11	4	1	–		

Table 2. Tumor distribution in 1,2-di-*n*-butylhydrazine dihydrochloride (1,2-DBH) treated and control Swiss mice

Group	Treatment	Effective No. and sex of mice	Animals with tumors of Lungs			Malignant lymphoma			Kidney			Other tissues[b]	
			No.	%	Age at death[a]	No.	%	Age at death[a]	No.	%	Age at death[a]		
1	0.0625% 1,2-DBH in drinking water daily for life	50♀	36	72	76 (47-98)	21	42	62 (21-93)	-	-	-	3 Angiomas in ovaries (69, 71, 79) 2 Angiosarcomas in livers (88, 93) 1 Angiosarcoma, subcutis (39) 1 Angioma in liver and ovary (60) 4 Adenocarcinomas of breasts (69, 70, 76, 77) 1 Fibrosarcoma, subcutis (81) 1 Adenocarcinoma of uterus (79) 2 Leiomyosarcomas of uterus (80, 87)	1 Adenocarcinoma of ovary (69) 1 Sq. cell papilloma of forestomach (55) 1 Sq. cell carcinoma of forestomach (63) 1 Polypoid adenoma of gl. stomach (93) 1 Granulosa cell tumor (77) 1 Sq. cell carcinoma of clitoral gland (55)
		50♂	38	76	72 (46-93)	6	12	65 (36-93)	4	8	75 (64-89)	1 Sq. cell carcinomas and papillomas of esophagus (78) 1 Angiosarcoma in subcutis, muscle and lymph node (56) 1 Angiosarcoma in liver (80) 1 Angioma in liver (68) 1 Fibroma, subcutis (83) 1 Sq. cell carcinoma of forestomach (69) 1 Papilloma of bladder (73)	1 Sq. cell carcinoma of preputial gland (78)
2	Untreated control	100♀	25	25	90 (29-141)	18	18	83 (16-128)	1	1	96	6 Angiomas in livers, uteruses and ovaries (60, 90, 98, 108, 110, 125) 4 Angiosarcomas in livers and uteruses (60, 82, 84, 111) 4 Papillomas of forestomachs (66, 90, 98, 101) 2 Adenocarcinomas of breasts (103, 120) 2 Fibrosarcomas, subcutaneous (79, 100) 1 Sarcoma, retroperitoneal (103) 1 Adrenocortical adenoma (117) 1 Granulocytic leukemia (91) 1 Myxosarcoma of uterus (105)	1 Granulosa cell tumor (49) 1 Polypoid adenoma of colon (84) 1 Fibrosarcoma of submandibular gland (112) 1 Adenocarcinoma of submandibular gland (141) 1 Fibroma of breast (114)
		100♂	26	26	86 (37-113)	8	8	81 (31-106)	-	-	-	9 Angiomas in livers (69, 69, 75, 87, 93, 98, 104, 115) 4 Angiosarcomas in livers (56, 82, 93, 97) 1 Fibrosarcoma, subcutaneous (91) 1 Adrenal cortical adenoma (93) 1 Adenoma of thyroid (99)	

[a] Average and range in weeks. [b] Age at death given in weeks in parentheses.

as adenomas and the other one as an adenocarcinoma. Grossly and histologically these tumors were identical to those described by other investigators[13,14]. Other tumors. In a number of instances other types of neoplasms were seen in the various groups shown in table 2. Since they occurred in low incidences, their appearances cannot be attributed to the treatment.

Discussion. The present findings demonstrate that lifelong administration of 0.0625% 1,2-DBH in drinking water to 6-week-old Swiss mice produced tumors of the lungs, lymphoreticular tissue, and kidneys. In treated females, the tumor incidences in these 3 tissues were 72 ($p > 0.000001$), 42 ($p > 0.003$), and 0% respectively, while in the treated males, they were 76 ($p > 0.000001$), 12 and 8% ($p > 0.03$), respectively. In untreated controls the corresponding tumor incidences were 25, 18 and 1% in females and 26, 8 and 0% in males. Statistical analysis was carried out by Fisher's exact probability test for 2×2 tables[15]. Histopathologically, the tumors were classified as adenomas and adenocarcinomas of the lungs, various types of malignant lymphomas, and adenomas and adenocarcinoma of kidneys. The current study is an integral part of our structure activity relationship inquiry. Specifically its aim is to reveal whether the disubstituted derivatives of hydrazines are more active carcinogens than the monosubstituted derivatives. In an earlier experiment *n*-butylhydrazine hydrochloride, a monoalkylhydrazine, administered as a 0.0125% solution in drinking water to Swiss mice induced only lung tumors[6]. The presently reported 1,2-DBH elicited the development of tumors of the lungs, lymphoreticular tissue, and kidneys. Therefore it seems evident that this dialkylhydrazine analogue is a stronger cancer-inducing agent than the corresponding monoalkyl derivative, since 1,2-DBH gave rise to tumors in 2 additional tissues at a lower dose. Previous investigations have clearly shown that symmetrical and unsymmetrical dimethylhydrazines are much more potent carcinogens than monomethylhydrazine[1-3]. Formylhydrazine, however, roughly exhibited tumors similar in type and incidence to those induced by 1,2-diformylhydrazine[4,5]. This study confirms the earlier findings that dialkylhydrazines are more potent carcinogens than the corresponding monoalkyl analogues. This does not appear to be true for mono- and disubstituted hydrazides however since formyl- and 1,2-diformylhydrazine exhibit similar activity. Further studies in this area are currently in progress. Hydrazines, hydrazides and hydrazones are known tumorigenic agents in laboratory animals. To date, well over 40 such compounds were shown to induce tumors in more than 2 dozen organs and tissues of mice, hamsters and rats[16,17]. The human population is exposed to approximately one-half of these chemicals in the form of drugs, agricultural herbicides, industrial chemicals and as naturally occurring ingredients of edible mushrooms and tobacco[18-23]. Therefore, this group should be considered an environmentally important class of chemicals.

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Ultrastructural study of patch-graft re-endothelialization[1]

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Summary. On the 8th post-operative day, an endothelial layer covers the 'venous patch' luminal part when the adventitia of the patch is sutured to a 5–8-mm-long arteriotomy performed in the common carotid of the rat. The probable origin of this secondary endothelium is discussed.

During the first post-operative hours, the endothelium of 'venous patch' grafts undergoes great alterations which lead to its disappearance by desquamation[2]. In areas where the endothelium has disappeared, the subendothelial connective tissue, especially the collagen fibres, is consequently in direct contact with the vascular lumen and becomes a thrombogenic surface where thrombocytes and fibrin can easily be deposited[3]. Great damage must occur in the endothelium in order to stimulate re-endothelialization[4]. The 'venous patch' re-endothelialization is completed during the 5th and 6th days when the endothelium of the venous patch is oriented toward the arterial lumen[2].

The controversy about the origin of secondary endothelial cells has not yet been solved. Some investigators have proposed that endothelial cells of the host vein may be responsible for the secondary endothelium (endothelial re-endothelialization)[4-13], while smooth muscle cells have been reported to originate secondary endothelial cells of the venous patch (muscular re-endothelialization)[14-22] and other studies[23-28] suggest that hematogenous re-endo-