Toxicological and Clinical Evaluation of a New Nitrogen Mustard, 2-[Di-(2-chloroethyl)-aminomethyl]benzimidazole*

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In the field of clinical cancer chemotherapy the alkylating agents are second only to androgens and estrogens in frequency of use. Since the first description of the effects of nitrogen mustard in problems of human neoplastic disease, congeners have been synthesized and tested in the laboratory and clinic. The objectives of this developmental program have been to increase anti-tumor specificity and to decrease toxicity. The latter goal has been attained to a limited degree as exemplified by a compound such as chlorambucil which is active following oral administration, produces less stimulation of the medullary vomiting center and less hematopoietic toxicity, but also is less effective therapeutically, so that the therapeutic index is approximately the same as that for the parent compound. The objective of tumor specificity has been even less certainly reached, although Myleran appears to be more effective on the chronic myelocytic leukemic process than on other human neoplasms (5).

Despite the meager encouragement which has rewarded the preparation and testing of more alkylating agents, the effort not only continues but grows apace. The present report summarizes another such study. In this investigation, however, a new policy for this laboratory and clinic regarding alkylating agents was established and implemented. This policy dictates that clinical trial of another representative of the alkylating drugs is justifiable only if laboratory data demonstrate that the compound is less toxic than other available congeners or has an anti-tumor action on a single tumor or a spectrum of tumors which is distinctly different from that of previously studied substances. Drugs of this class so selected for clinical trial, the policy continues, shall not be evaluated against those human diseases known to respond to alkylating agents but rather shall be given the more exacting trial of being tested against tumors not responsive to currently available compounds. It is believed that adherence to this policy will reduce the frequency of clinical trials of alkylating agents and will guide the human studies to the most important issue, namely, whether the new drug differs qualitatively from those now in use.

Recently, Hirschberg, Gellhorn, and Gump (4) described the synthesis of a new nitrogen mustard (benzimidazole mustard; 2-[di-(2-chloroethyl)aminomethyl]benzimidazole hydrochloride) (Chart 1). Studies of the activity of the compound in a variety of experimental tumors demonstrated effects which clearly distinguished it from others studied (3). These laboratory findings appeared to justify further investigation, and the present report is a summary of some toxicological data in rabbits and dogs and of the results of the clinical trial of this compound.

MATERIALS AND RESULTS

Administration of benzimidazole mustard to rabbits and dogs.—For most of the animal experiments, benzimidazole mustard was dissolved at concentrations of 0.5 or 1.0 mg/ml in a solution consisting of physiological saline and water (1:1). In a few instances, 30 per cent propylene glycol was used as the solvent. The daily dosage levels ranged from 1.0 to 15.0 mg/kg in rabbits and from 1.0 to 6.0 mg/kg in dogs, and the number of injections from one to sixteen given over 1–22 days. The agent was injected into the marginal vein of the ear of the rabbits and into the femoral vein of the dogs, unless otherwise indicated.

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Table 1 summarizes the changes in body weight, hemoglobin, and leukocyte and erythrocyte counts in ten rabbits, ranging from 1.2 to 2.6 kg. in weight, after administration of benzimidazole mustard in saline. Observations were made almost daily throughout the course of treatment, but

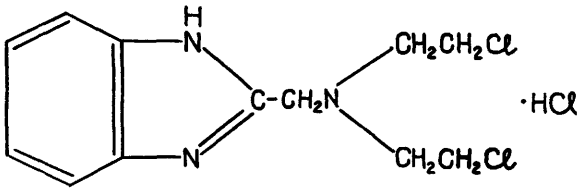

CHART 1.—Structural formula of benzimidazole mustard, 2-[di-(2-chloroethyl)aminomethyl]benzimidazole hydrochloride.

general, a drop in the leukocyte count was the most significant index of toxicity. Differential counts were carried out frequently but were noncontributory and, therefore, are not presented here.

In view of the relatively slight solubility of the agent in saline, fairly large volumes of solution had to be injected to achieve the higher dosage levels. It was of interest, therefore, to test the toxicity of the agent in 30 per cent propylene glycol, in which concentrations of 5 mg/ml are easily achieved. In a preliminary experiment with two rabbits, it was shown that this solvent, administered on thirteen occasions during a 19-day period, was completely nontoxic. When, however, benzimidazole mustard was injected intraperitoneally at 15 mg/kg in this solvent into two rabbits,

TABLE 1

EFFECT OF BENZIMIDAZOLE MUSTARD IN SALINE ON RABBITS

Rabbit no.	Days of observation	Dosage schedule		Initial and final				Day terminated
		Mg/kg OD	No. doses	Body weight	Hemoglobin	RBC	WBC	
				(kg.)	(gm. per cent)	($\times 10^6$)	($\times 10^3$)	
1	1–17	1.0	12	1.60–1.61	12.5–12.0	6.0–4.4	14.7– 8.5	17–Sacrificed
2	a) 1–19	1.0	15	2.19–1.98	15.5–14.0	6.9–6.3	12.6–10.3	
	b) 22–33	2.0	9	2.06–2.05	15.0–14.0	6.6–4.8	8.2– 5.9	
	c) 36–40	4.0	4*	1.96–2.01	13.5–12.5	6.8–5.3	11.1–10.7	40–Sacrificed
3	a) 1–19	1.0	15	2.07–1.90	12.5–12.0	5.7–5.5	15.3–10.3	
	b) 22–33	2.0	9	1.98–1.91	13.5–13.5	6.6–5.3	8.9–12.1	
	c) 36–39	4.0	4*	2.00–1.92	14.0–14.0	5.7–5.9	15.8–14.7	39–Sacrificed
4	1– 8	1.5	6	1.46–1.22	14.0–12.5	6.6–4.1	12.0– 9.8	8–Died
5	1–12	1.5	10	1.22–0.94	14.0–11.5	6.5–4.8	7.2–10.3	14–Died
6	1–7	4.0	5	2.61–2.26	14.5–14.0	5.9–5.7	17.4– 9.8	7–Died
7	a) 1–22	4.0	15	2.12–2.04	13.5–11.5	5.2–5.6	9.3– 7.7	
	b) 23–37	6.0	10	2.03–1.91	12.0–12.5		8.2– 6.5	
	c) 40–58	12.0	15	1.89–1.64	14.0–13.0		5.7– 3.5	60–Died
8	a) 1–22	4.0	16	1.52–1.56	12.5–12.0	4.6–4.9	15.3– 4.2	
	b) 23–37	6.0	10	1.56–1.58	12.5–13.0		5.3– 5.3	
	c) 40–61	12.0	14	1.57–1.30	16.0–10.5		7.6– 3.8	61–Sacrificed
9	a) 1–10	12.0	7	1.72–1.63	13.5–13.0	6.4–6.3	6.1– 5.2	
	b) 13–16	15.0	3*	1.54–1.51			4.1– 3.5	16–Sacrificed
10	a) 1–10	12.0	7	2.58–2.32	12.0–12.5	6.2–5.7	7.4– 5.0	
	b) 13–16	15.0	3*	2.29–2.02			5.4– 5.5	16–Sacrificed

* Intraperitoneal injections.

this summary of the differences between initial and final values for each treatment period provides an adequate picture of the over-all effect. The wide variation in response among these animals was a striking feature of the experiment and gave an indication of the unpredictability of toxic reactions to this nitrogen mustard derivative. In one died with convulsions within 90 minutes after a single injection, and the second became weak and eventually comatose after the first injection and succumbed within a half hour of the second injection on the following day.

Table 2 records the response of seven dogs, ranging from 4.7 to 7.5 kg. in weight, to the

administration of various dosage levels of the agent. An additional dog given a single 4 mg/kg dose of the nitrogen mustard derivative in 30 per cent propylene glycol died within 45 minutes of the injection after exhibiting marked weakness and convulsions. The toxicity in dogs is somewhat greater than that in rabbits or mice (4); in dogs, too, the drop in leukocyte count is the most sensitive index of toxicity.

Complete autopsies were carried out on rabbits Nos. 1–3 and 7–10 and on dogs Nos. 1, 2, 4, and 7. There were no significant gross abnormalities, and histological examination of the various tissues (liver, heart, lung, spleen, kidney, bladder, muscle, pancreas, brain, small and large intestine, stomach, testis, bladder, rib, and sternum) was entirely noncontributory.

Clinical evaluation of benzimidazole mustard.— Twenty-seven patients have been treated with the benzimidazole mustard. All the patients who were included in the study had far advanced neoplastic disease. The diagnosis in each patient was established by history, physical examination, and appropriate laboratory tests including biopsy, and the data are shown in Tables 3 and 4. Complete blood cell counts and platelet counts were made before the start of therapy and at regular intervals thereafter. Liver and renal function tests were performed at appropriate intervals. In fourteen patients, serial electroencephalograms were also performed.

For intravenous administration, the benzimidazole mustard was dissolved in sterile saline in a concentration of 2.0 mg/ml. Single doses of from 5 to 150 mg. were injected without further dilution. Table 3 summarizes the pertinent information on eleven patients who received one to three courses of benzimidazole mustard intravenously. The total dose on a body weight basis ranged from 0.6 to 8.0 mg/kg/course administered in from 1 to 5 days, while three patients who had more than a single course received a total cumulative dose ranging from 4.5 to 10.0 mg/kg. None of these patients showed any therapeutic benefits. There were no signs of local skin irritation, phlebitis, or thrombosis at the site of injection. In two patients, there were nausea and vomiting shortly after the injection of the benzimidazole mustard. Another patient was observed to have a grand mal seizure 1 hour after receiving the fourth dose of benzimidazole mustard. Patients receiving less than 5.3 mg/kg, total dose, did not develop late hematopoietic depression. Three patients (Table 3, cases 9, 10, and 11) developed significant changes in their blood picture, consisting of leukopenia and thrombopenia 2–3 weeks after the start of therapy. The hematopoietic depression continued for at least 10 days, after which the blood count returned to normal. There was no evidence of renal or of hepatic toxicity in any of the patients.

Benzimidazole mustard was administered orally

TABLE 2

EFFECT OF BENZIMIDAZOLE MUSTARD IN SALINE ON DOGS

Dog no.	Days of observation	Dosage schedule		Initial and final				Day terminated
		mg/kg OD	No. doses	Body weight	Hemoglobin	RBC	WBC	
				(kg.)	(gm. per cent)	($\times 10^6$)	($\times 10^3$)	
1	a) 1– 4	1.0	3	5.5–5.2	13.5–14.0	6.9–5.9	11.1–.8.4	
	b) 7–18	2.0	5	5.3–4.6	13.0–12.5	3.3–5.9	6.3– 5.0	
	c) 21–25	4.0	2	4.2–3.5			5.1– 3.6	25–Sacrificed
2	a) 1– 4	1.0	4	5.0–4.2	17.0–16.0	7.1–5.0	21.6–10.2	
	b) 7–11	2.0	4	3.9–3.4	15.5–14.0	4.5–6.5	9.8– 6.8	16–Sacrificed
3	a) 1– 5	2.0	4	6.5–5.6	13.5–13.0	6.2–6.4	9.8– 7.3	
	b) 8	4.0	1	4.6	13.5	6.2	6.5	11–Died
4	a) 1– 5	2.0	4	6.0–5.6	14.0–12.5	6.6–6.5	10.0– 7.8	
	b) 8–18	4.0	8	5.1–4.0	12.0–11.5	6.3–3.7	6.6– 4.0	19–Sacrificed
5	1– 5	4.0	3	5.2–4.7	14.0–13.5	6.3–6.3	14.1– 6.5	6–Died
6	1– 6	4.0	4	4.7–4.0	14.5–16.0	6.4–6.2	14.3– 5.2	6–Died
7	a) 1–18	4.0	12	7.5–5.8	16.0–13.0	6.4–5.4	10.0– 4.0	
	b) 21–24	6.0	3	5.9–5.3	13.0–12.5	5.4–4.9	5.5– 1.7	25–Sacrificed

TABLE 3

DATA ON PATIENTS TREATED WITH INTRAVENOUS BENZIMIDAZOLE MUSTARD*

CASE	SEX/AGE	DURATION OF DISEASE (mos.)	PREVIOUS THERAPY	DRUG DOSAGE				HEMATOLOGIC RESPONSE			PRESENT STATUS FROM START OF TREATMENT
				Mg. OD	Days	Total dose	Mg/Kg	WBC ×10³	Plts. ×10³	Toxicity	
1. Ca kidney	M/54	2	Radiotherapy	5	6	30	0.6	6.4/6.4	270/120	V	Died 1 mo.
2. Ca lung	M/54	10	Radiotherapy	10	5	50	0.8	No change	No change	None	Died 15 days
3. Ca lung	M/71	1	Radiotherapy	10–12	5	52	0.8	No change	440/270	None	Died 17 days
4. Ca lung	M/59	14	Radiotherapy	45	1	45	1.0	No acute toxicity after a single dose			Died 2 days
5. Ca lung	M/56	1	Radiotherapy	150	1	150	3.0	No acute toxicity after a single dose			Died 2 days
6. Carcinomatosis	M/72	1	Radiotherapy	a) 15	5	75	1.5	12.0/8.0	270/20	None	
				b) 30	5	150	3.0	8.7/5.9	270/160	None	Died 1 mo.
							4.5				
7. Ca lung	F /67	36	Radiotherapy	120	1	120	2.0	4.2/2.8	212/186	N, V	Alive 6 mo.
8. Ca lung	M/73	3	Radiotherapy	a) 15	5	75	1.7	8.0/8.0	200/180	None	
				b) 37	5	162	3.6	8.0/7.0	180/180	None	Died 1 mo.
							5.3				
9. Glioblastoma multiforme	F/50	—	Radiotherapy	a) 50	1	50	1.0	13.0/13.0	180/182	None	
				b) 50	3	150	3.0	13.0/9.1	180/190	None	
				c) 100	3	300	6.0	9.1/1.2	190/20	L, T	Normal CBC 4 mo.
							10.0				
10. Ca lung	M/73	2+	Radiotherapy	120	4	472	8.0	12.3/4.7	342/124	None	Alive 23 mo.
11. Ca colon	F /61	84	Surgery	100	4	400	8.0	8.7/0.5	520/10	L, T and GM Seizure	Died 1 mo. WBC ok. Plts. 70,000

* Explanation of abbreviations, see footnote Table 4.

TABLE 4

DATA ON PATIENTS TREATED WITH ORAL BENZIMIDAZOLE MUSTARD *

CASE	SEX/AGE	DURATION OF DISEASE (mos.)	PREVIOUS THERAPY	DRUG DOSAGE				HEMATOLOGIC RESPONSE			PRESENT STATUS FROM START OF TREATMENT
				Mg. OD	Days	Total dose	Mg. / Kg	WBC $\times 10^3$	Plts. $\times 10^3$	Toxicity	
1. Glioblastoma multforme	M/40	9	Surgery	a) 300 b) 100	1 1	400	8.0	9.5/17.5	500/490	V	Died 1 mo.
2. Reticulum sarcoma	M/57	6	Radiotherapy	200	2	400	8.0	9.5/1.3	278/22	N, V, L, T, C	Alive 1.5 mo.
3. Ca lung	F/61	19	Radiotherapy	200	2	400	9.1	10.0/14.0	360/116	None	Died 7 days
4. Ca lung	M/58	1	None	200	4	800	10.0	14.0/5.0	210/130	N, V Hallu-cina-tions	Died 18 days
5. Mesothelioma	M/37	4	Radiotherapy ThioTEPA	200	4	800	16.0	12.6/0.2	372/10	L, T, C, N, V	Died 25 days PM—Necrotic tu-mor mass
6. Ca colon	M/71	1	Surgery	300	4	1200	24.0	12.0/0.05	268/64	L, T, C	Died 5 days
7. Reticulum sarcoma	M/53	24	Radiotherapy HN2	a) 25 b) 25	3 2	75 50					
						125	2.0	8.7/8.6	42/80	N, V	Died 14 days
8. Reticulum sarcoma	M/48	8	Radiotherapy HN2	a) 25 b) 25	3 2	75 50					
						125	2.0	8.8/8.6	140/88	N	Died 1 mo.
9. Lymphocytic lymphosar-coma	M/49	24	Radiotherapy CB-1348	25	8	200	3.5	8.2/6.9	88/20	T	Died 14 days
10. Hodgkin's disease	M/64	8	Radiotherapy HN2 CB-1348	a) 50 b) 25	1 5	50 125					
						175	3.0	11.4/0.6	160/10	L, T	Died 2 mos.
11. Abdominal carcinomat.	F/19	7	Radiotherapy HN2	25	5 (3 weeks)	125	3.2	11.0/6.0	240/220	N	Living 9 mos.

* Abbreviations: M—male; F—female; Mos.—months; ThioTEPA—Triethylenethiophosphoramide; HN2—nitrogen mustard; CB-1348—chlorambucil; Mg. OD—Mg/day; WBC—white blood cells $\times 10^3$ per cu. mm.; Plts—platelets $\times 10^3$ per cu. mm.; N—nausea; V—vomiting; L—leukopenia; T—thrombocytopenia; C—clinical manifestations of hematopoietic toxicity; GM seizure—grand mal seizure; and PM—post mortem examination. Blood counts are given at the beginning and the end of therapy.

TABLE 4—*Continued*

Case	Sex/Age	Duration of disease (mos.)	Previous therapy	Drug dosage				Hematologic response			Present status from start of treatment
				Mg. OD	Days	Total dose	Mg./Kg.	WBC $\times 10^3$	Plts. $\times 10^3$	Toxicity	
12. Glioblastoma	F/45	12	Surgery Radiotherapy	25	7	175	3.0	9.5/5.3	140/124	N, V	Died 4 mos.
13. Glioblastoma	M/38	8	Surgery Radiotherapy Steroids	a) 25 b) 100	5 1	125 100					
						225	4.2	8.7/9.5	270/232	N, V	Died 2 mos.
14. Glioblastoma	F/58	6	Radiotherapy	25	5	125	2.0	6.1/6.8	No change	None	Living 5 mos.
15. Glioblastoma	M/63	3	Radiotherapy	25	5	125	1.8	8.7/6.0		N, V	Lost to follow-up after 2 mos.
16. Glioblastoma	F/40	10	Radiotherapy	25	6	150	5.0	11.5/8.8		N, V	Died 21 days

to six patients in capsules containing 100 mg. of the powder. The drug was administered in the fasting state, and the patients were not allowed to eat for at least 2 hours. Single doses of from 100 to 300 mg. were administered. Table 4 summarizes the information on these six patients. The total dose on a body weight basis ranged from 8.0 to 24.0 mg/kg, administered over 2–4 days. Two patients showed temporary objective improvement (Table 4, cases 2 and 5). In four of the six patients, administration of each dose was followed in a few hours by marked nausea and severe spells of vomiting, alleviated, in part, by large doses of chlorpromazine. One patient (Table 4, case 4) had severe visual hallucinations, relieved by barbiturates. Four patients of this group had electroencephalograms before and after administration of benzimidazole mustard. No significant changes were observed in any of these. Three of the six patients showed severe hematopoietic toxicity. One of these patients (Table 4, case 2), 11 days after the start of therapy, showed marked objective improvement with significant decrease of cervical adenopathy and abdominal masses as well as relief of dyspnea. This improvement, however, lasted only 3 weeks, and, by the time the blood picture reached normal levels, the patient's disease had also returned to the pre-treatment status.

Benzimidazole mustard was given orally in smaller doses to ten patients, in capsules containing 25 mg. of the powder. Table 4 summarizes the pertinent data on ten patients who received 25 mg. daily to 50 mg/week of the oral benzimidazole mustard (cases 7–16). The total dosage ranged between 125 and 225 mg., or 1.8–5.0 mg/kg body weight given for 5–7 days. In seven patients, there appeared mild to moderate nausea and vomiting following administration of the drug, but not severe enough to warrant discontinuation of therapy. None of the patients showed untoward neuropsychiatric reactions, and the electroencephalograms did not change in any patient. One patient (Table 4, case 10) showed severe hematopoietic toxicity. This patient, with wide-spread Hodgkin's disease, had extensive radiotherapy and treatment with other alkylating agents prior to the trial of benzimidazole mustard. Severe leukopenia and thrombopenia accompanied by purpura developed 10 days after the start of therapy and persisted for 24 days. Two other patients (Table 4, cases 8 and 9) developed some thrombopenia but showed no clinical toxicity. One patient (Table 4, case 11) showed definite objective improvement with decrease in size of metastatic skin nodules.

The compound discussed in the present report

was synthesized and brought to clinical trial for the purpose of evaluating the activity of a bifunctional alkylating moiety, attached to a weak purine antagonist, in animals and patients. The present investigation has shown that the toxicity of this agent in man far outweighs the therapeutic benefits. The gastrointestinal and central nervous system toxicities may be due in part to the mustard moiety (6) but in part also to benzimidazole, which has been reported to have central effects (1, 2). When tested against a variety of malignant diseases which do not respond to the parent compound, this new nitrogen mustard also gave no objective evidence of benefit.

SUMMARY

Benzimidazole mustard (2-[di-(2-chloroethyl)-aminomethyl]benzimidazole hydrochloride) was tested in animals and man to evaluate its toxicity. It was then studied in patients with neoplastic diseases usually refractory to nitrogen mustard to determine distinctive therapeutic efficacy. Nausea, vomiting, central nervous system stimulation, and depression of hematopoiesis occurred with large doses without apparent therapeutic benefit.

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