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Reversal of Nitrogen Mustard Intoxication by a Serotonin Antagonist.* (27688)

JOHN B. FIELD, ANNIE MIRELES AND EDWARD C. DOLENDO
Institute for Cancer and Leukemia Research, Culver City, Calif.

A serious limitation to the effective clinical usefulness of many anti-cancer drugs has been the destruction of bone marrow and associated complications. With the use of epinephrine and nor-epinephrine it now appears possible selectively to protect both animals(1) and human beings(2,3) from the lethal and marrow-depressing effects of alkylating agents (nitrogen mustard). Further studies of this observation have occupied this laboratory. The present report indicates a unique newer direction evolving from this program.

The effects of epinephrine in mice in preventing death and ameliorating leukopenia were found to be slight but the optimum activity occurred when the epinephrine was administered several hours after the alkylating agent had been given. During this study it was found that a serotonin antagonist possessed a significant ability to reduce the consistent leukopenia induced by the nitrogen mustard and also to reduce the anticipated mortality produced by the nitrogen mustard. Not only did the anti-serotonin agent exhibit this effectiveness before nitrogen mustard was given but particularly the optimum response appeared when it was given several hours after the alkylating agent.

Method. Mechlorethamine (nitrogen mustard) (HN_2) was freshly prepared in saline solution† and given intraperitoneally to rested adult White Swiss female mice 18-22 g in weight in a single dose of 5 mg/kg. This amount produced a virtually uniform 94-100% mortality by the sixth day. The serotonin antagonist, 1-(N-methyl-piperidyl-4')-3 - phenyl - 4 - benzyl - pyrazolone - 5 (KB-95)‡ was prepared in suspension in 5% gum acacia solution and was given intraperitoneally in a dose of 400 mg/kg to groups of mice at intervals of ½ hr before and 2, 8 and

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‡ Furnished by Mr. Henry Althouse of Sandoz, Inc.

TABLE I. Effect of HN_2 Alone or HN_2 Followed by KB-95 on Survival and Body Weight of Mice.

	Control mice (HN_2 alone)	KB-95 treated mice* (HN_2 plus KB-95)			
		½ hr before	2 hr after	8 hr after	24 hr after
Surviving mice	5/78 (6%)	6/14 (43%)	17/22 (77%)	9/12 (75%)	11/14 (79%)
			Body wt, g		
Day 1	20.2	22.0	20.4	21.8	20.0
2	18.9	19.8	19.4	20.6	18.3
3	17.8	18.5	19.3	19.8	18.2
4	16.3	17.5	17.9	18.6	17.9
5	16.1	17.1	16.9	18.4	18.2
6	15.9	16.5	16.0	18.0	18.8
7	15.0	16.2	16.3	18.5	22.5

* KB-95 in all cases was given in single dose I.P. at stated time after single administration of HN_2.

24 hours after the HN_2 was given. Daily blood samples taken from the tip of the tail for the white cell counts and daily body weight were recorded for all mice. Control groups of mice received only the HN_2 or the KB-95.

Results. In Table I are given the survival and weight data. It is apparent that the pyrazolone compound (KB-95) produced not only a reduction of the lethal effect of the HN_2 when given before the HN_2, but also when given after it. There was a survival of 75 to 79% when the KB-95 was given 2 to 24 hours after the HN_2. This was consistently reproducible in many tests. The weight of the KB-95 treated mice increased after the serotonin antagonist was given 8 and 24 hours after the HN_2 whereas the control HN_2 mice steadily lost weight.

In Table II are given the data on white blood cell counts. White cell counts of the control HN_2-treated mice steadily fell, to about 2000/cmm or less before death. When KB-95 was given just before or after the HN_2, a considerable leukopenia was observed but this never fell to the low levels of the control mice. It is interesting to observe in both the 8- and 24-hour KB-95 treated groups an initial fall in wbc to levels resembling those of the control mice, but within 48 hours a significant reversal occurred with the fall in the count plateauing and then returning to pre-treatment levels. Normal white blood cell levels were almost always reattained about 5 days after the KB-95 was given. The mice surviving the administration of HN_2 when treated with KB-95 have been observed for several months. No further deaths which could be attributed to the HN_2 were seen and the white blood cell count remained constant.

TABLE II. Effect of HN_2 Alone or HN_2 Followed by KB-95 on Average Leukocyte* Count of Mice.

Day	Control mice (HN_2 alone)	KB-95 treated mice† (HN_2 plus KB-95) ½ hr before	2 hr after	8 hr after	24 hr after
	per mm³				
1	4886	5187	4625	3995	5456
2	3131	4345	4567	4306	3935
3	2241	2120	4570	4625	5862
4	2221	2562	6108	5398	9212
5	2569	3462	8409	6625	9470
6	2666	6675	10,603	7831	10,070
7	—	7500	11,987	9015	10,589

* Leukocyte count is avg of all mice.

† KB-95 given I.P. in one dose at stated time after HN_2 was given.

Discussion. It appears clear that at least in the mouse, the lethal and leukopenia-inducing effects of the alkylating agent, HN_2, can be consistently prevented by the pyrazolone agent (KB-95). The latter has been demonstrated to be a potent serotonin antagonist§ but whether the serotonin-epinephrine interplay is involved in the present finding remains to be investigated. It is assumed, however, that the restoration of normal white blood cell levels also implies a return to normal function of a nitrogen mustard-disturbed bone marrow. The events observed make this appear reasonable.

The means to reverse toxic and lethal phar-

§ Unpublished data from laboratories of Sandoz, Inc.

macological reactions are eagerly sought. It would seem to be of considerable biochemical interest to attack a lethal process already well underway and to nullify and reverse it. The reversal of the lethal action of nitrogen mustards in mice appears to be such an example. It would seem that fruitful and perhaps practically significant clinical findings await further clarification of the interrelationships suggested here.

Summary. The intraperitoneal administration of a pyrazolone compound before and after a single intraperitoneal nearly-lethal dose of nitrogen mustard had been given to mice produced a marked reduction of the mortality expected from the nitrogen mustard. Furthermore the leukopenia resulting from the nitrogen mustard was considerably less in extent and duration.

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2. Field, J. B., *Clin. Research,* 1961, v9, 103.
3. ———, *Proc. Am. Assn. Cancer Research,* 1961, v5.

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Renal Effects of Renin in Normal and Buffer-Nerve Sectioned Dogs.* (27689)

F. DEL GRECO,† A. C. CORCORAN‡ AND I. H. PAGE
Research Division, Cleveland Clinic Foundation, Cleveland, Ohio

The effect of renin on urine flow and composition is not as well defined in the dog as in other laboratory animals(1). Previous studies in normal dogs and in those with diabetes insipidus have shown that following renin injection urine flow increases(2), decreases(3, 4) or does not change(5). These disparate observations are probably attributable to differences in experimental procedures. The present study was therefore undertaken to reexamine the effects of renin in normal dogs under standardized conditions. The condition selected was that of osmotic (mannitol) diuresis during hydropenia since water and electrolyte excretion tends, in this situation, to be relatively stable(6). Then, since section of the carotid sinuses and aortic depressor nerves in dogs (abbreviated below as buffer-nerve sectioned dogs) enhances the pressor response to renin and angiotensin(7), similar experiments were performed in dogs so prepared.

Material and methods. Ten normal and 6 buffer-nerve sectioned dogs, weighing from 6 to 20 kg, were used. The latter dogs were studied at least one month after section of the buffer-nerves, and after daily measurements of blood pressure in the resting, unanesthetized state had shown sustained hypertension.

The effects of renin were determined in all the animals after induction of anesthesia by intravenous sodium pentobarbital (15 to 30 mg/kg/B wt), in the course of osmotic (mannitol) diuresis and hydropenia. The procedures employed have been described(8).

Three to 5 consecutive control clearance periods were obtained at 10-15-minute intervals. Renin,§ diluted in the infusion fluid used for the study of kidney function, was then administered at a constant rate. The urine collected during the first 5 to 10 minutes of renin infusion was discarded. Subsequently, 3 to 5 consecutive clearance periods were determined. After the administration of renin was discontinued and after a 5-10-minute discard period, another 3 to 5 clearance periods were again determined.

Throughout the experiments mean blood

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† Present address: Passavant Memorial Hospital, Chicago, Ill.

‡ Present address: St. Vincent Charity Hospital, Cleveland, Ohio.

§ Hog renin, partially purified by several ammonium sulfate precipitations and assayed according to Goldblatt *et al.*(9), contained 30 pressor units (P.U.) per ml.