Potassium Iodide Interaction with Cyclophosphamide in Mice (35630)

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Williams (1) recently reported the use of potassium iodide (KI) to increase the effectiveness of cyclophosphamide (Cytoxan, NSC-26271, *N*, *N*-bis (β-chlorethyl)-*N'*, *O*-propylene phosphoric acid ester diamide monohydrate) in patients with Burkitt's tumor. Cyclophosphamide is an antineoplastic agent of the nitrogen mustard class which requires activation by hepatic microsomal drug-metabolizing enzymes (2, 3). The use of KI is well established in several medical conditions, *e.g.*, asthma and thyroid disease, but it has not been previously reported as being helpful in the treatment of neoplasms.

Due to the clinical interest in this drug combination it was felt that a laboratory investigation of the interaction between KI and cyclophosphamide would be valuable.

Materials and Methods. CDF_1 male mice were used. Some of the mice were injected intraperitoneally with L1210 leukemia cells obtained from CDF_1 donor mice.

Cyclophosphamide solution was injected intraperitoneally (ip) and the KI solution was injected subcutaneously (sc) in the posterior part of the neck. All drug solutions were prepared using sterile water immediately prior to the experiment, and all drugs were used within 1 hr of their preparation. Mice received 0.01 ml of drug solution per gram of body weight.

Sleeping times were determined following hexobarbital (Evipal, Winthrop) (100 mg/kg) administered intraperitoneally. The time, in minutes, until the animal regained its righting reflex was used to estimate sleep.

Doses lethal to 50% of the mice and potency ratios were calculated according to the method of Litchfield and Wilcoxon (4).

Results. Table I presents the lethality of KI to CDF_1 male mice after subcutaneous injection. All mice receiving the highest dose died within 24 hr of treatment, presumably due to cardiac toxicity. There were no significant gross abnormalities at autopsy.

TABLE I. Lethality of KI for CDF_1 Male Mice.[a]

Dose (g/kg sc)	Number dead/number tested
2.00	10/10
1.80	2/10
1.60	1/10
1.50	0/10
1.30	0/10
1.10	0/10

[a] Mice were followed 30 days.

The LD_{50} is approximately 1.90 g/kg. Therefore, all doses used in experimental animals were selected to fall far below the lethal level of KI alone.

The effect of potassium iodide on the lethality of cyclophosphamide is presented in Fig. 1. Two groups of mice were pretreated subcutaneously with either normal saline or KI solution (200 mg/kg). Thirty minutes later, each group received cyclophosphamide administered intraperitoneally at doses ranging from 200 to 800 mg/kg. The potency ratio is 1.40 (4) which is statistically significant. Therefore, it can be seen that pretreatment with KI solution significantly increased the lethality of cyclophosphamide.

The antitumor effect of KI was also studied. Mice were inoculated intraperitoneally with L1210 leukemia (10^5 cells) on day 0, and then treated on day 2 with either sterile saline or KI at doses of 100, 200, or 800 mg/kg. Table II demonstrates that KI treatment has no effect on the survival times of mice with L1210 leukemia.

The possible therapeutic interaction of KI

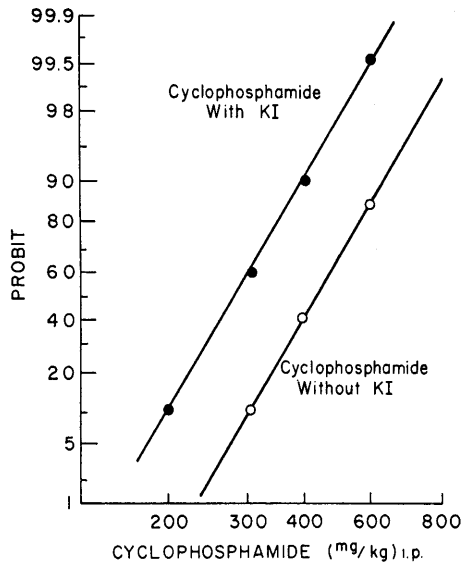

FIG. 1. Effect of KI on lethality of cyclophosphamide. There were 25 mice in each group. The curves are parallel and the difference is significant (4). The dose of KI was 200 mg/kg subcutaneously. Mice were followed for 30 days.

on the antitumor effect of cyclophosphamide was also investigated. Table III presents these data. It can be seen that KI pretreatment produced no synergistic effect using therapeutically effective doses of cyclophosphamide. However, there was a slight increase in survival time at the lower dose of cyclophosphamide although the difference was not significant.

To further test the interaction of KI and cyclophosphamide, inocula of 6×10^3–6×10^6 L1210 leukemic cells were injected intraperitoneally on day 0. On day 2, Cytoxan, 250 mg/kg, with or without KI, 200 mg/kg, was administered. Table IV presents these results. There was no increased survival in those mice pretreated with potassium iodide when compared with mice treated with cyclophosphamide alone.

TABLE II. Lack of Therapeutic Effect of KI on L1210 Leukemia on CDF_1 Male Mice.[a]

Treatment	Mean day of death
Normal saline	7.6
KI 100 mg/kg	7.8
KI 200 mg/kg	7.8
KI 800 mg/kg	7.7

[a] Mice were inoculated ip with L1210 leukemia (10^5 cells) on day 0 and treated once subcutaneously on day 2. There were 20 mice in each group. There was no significantly increased ILS (increased life survival).

The mechanism of the increased lethality of cyclophosphamide after potassium iodide pretreatment is unclear. Knowing that cyclophosphamide is activated by hepatic microsomal enzymes (3), it seemed possible that this system might be altered by KI. We attempted to study this *in vivo* using hexobarbital sleeping times in the mice.

TABLE III. Therapeutic Effect of KI on L1210 Leukemia with Cyclophosphamide.[a]

Cyclophosphamide (mg/kg, iv)	KI (mg/kg, sc)	Mean day of death
125	0	14.1
125	200	17.4
250	0	20.3
250	200	18.6

[a] Each group contained 10 mice. The inocula of L1210 leukemic cells was 10^6 in each case.

Mice were injected with KI (100 mg/kg) subcutaneously 10 and 60 min before the hexobarbital treatment. Another group was injected daily with KI for 3 days prior to the barbiturate. The mice were then injected intraperitoneally with 100 mg/kg of hexobarbital and observed closely for return of the righting reflex. These results are shown in Table V. There was no significant change in the sleeping time of mice after pretreatment with KI which suggests no change in the microsomal enzyme system.

Discussion. The clinical report of Williams (1) suggesting that KI increased the therapeutic effectiveness of cyclophosphamide in the treatment of Burkitt's tumor prompted us to investigate this interaction further using experimenal animals. Studies were designed to determine whether it would be possible to increase in mice the toxic and/or therapeutic effect of cyclophosphamide with prior KI treatment.

The results of these studies indicate that KI pretreatment increased the lethality of cyclophosphamide in mice but failed to alter

TABLE IV. Effect of KI on the Antitumor Effect of Cyclophosphamide Using Varying Inocula Of L1210 Leukemia.[a]

Number of cells inoculated, ip	Mean day of death		
	Saline control	Cyclophosphamide (250 mg/kg, ip)	Cyclophosphamide (250 mg/kg, ip) + KI (200 mg/kg, sc)
6×10^6	8.2	16	16
6×10^5	8.6	18	14
6×10^4	9.6	8/10 survived	7/10 survived
6×10^3	11.5	10/10 survived	8/10 survived

[a] Each group contained 10 mice. Mice were followed for 30 days.

TABLE V. Sleeping Times In Mice.[a]

Pretreatment time	Sleeping times (min)	
	Control (saline, 0.01 ml/mg body wt)	Experimental (KI, 200 mg/kg sc)
10 min prior	24.6 ± 2.11	23.8 ± 1.39
60 min prior	18.4 ± 2.42	23.4 ± 5.21
Daily × 3 days	27.3 ± 2.21	27.6 ± 2.01

[a] There were 10 mice per group. Values indicate mean plus or minus standard deviation.

hexobarbital sleeping times. KI pretreatment also failed to increase the therapeutic effectiveness of cyclophosphamide for mice inoculated with L1210 leukemia. Therefore, these data fail to demonstrate a significant therapeutic synergistic effect of KI for cyclophosphamide. The mechanism by which KI increased cyclophosphamide lethality is unclear, but does not appear to involve changes in microsomal drug metabolism as indicated by the failure of KI pretreatment to alter hexobarbital sleeping times.

These data do not exclude the possibility that KI could have a synergistic effect at some other dosage range and/or schedule. Further experiments might show some other combined effect.

Summary. Potassium iodide (KI) pretreatment, which has been reported to increase the clinical effectiveness of cyclophosphamide, increased the lethality of cyclophosphamide for mice. However, KI pretreatment failed to increase the experimental therapeutic effectiveness of cyclophosphamide for mice inoculated with L1210 leukemia. KI pretreatment also failed to alter hexobarbital sleeping times. There appears to be no significant synergistic action of KI on the therapeutic effects of cyclophosphamide investigated.

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