Influence of Pokeweed Mitogen, Phytohemagglutinin, and Bacillus Calmette-Guérin on the Toxicity of Rubidomycin on DBA/2 Mice[1] (37652)

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(Introduced by J. Kieler)

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The phytomitogens phytohemagglutinin (PHA) and pokeweed mitogen (PWM) induce transformation of human lymphocytes from the peripheral blood *in vitro* (1, 2) and morphological changes in the lymph nodes and spleen of rodents when administered *in vivo* (3–5). Bacillus Calmette-Guérin (BCG) is able to transform lymphocytes from sensitized persons *in vitro*, and morphological changes are seen in the lymphoid system of mice after repeated treatment with BCG *in vivo* (5). Furthermore a number of animal experiments indicate that phytomitogens may have an antineoplastic effect (6, 7). In addition to this, PHA and PWM have been shown to exert radioprotective effects (8, 9). These observations call for further studies of the combined effect of phytomitogens and radiomimetic antineoplastic compounds.

Alternating or simultaneous chemotherapy and nonspecific immunotherapy against malignant disorders is being tried at several centers (10). Encouraging but so far nonconclusive therapeutic results are continuously being reported, but very little is known about the influence of immunotherapy on the toxic side effects of cytostatic agents. The present study was undertaken in order to elucidate the influence of two phytomitogens (PHA and PWM) and BCG on the toxic effects of rubidomycin. We chose rubidomycin because it is a potent cytostatic agent used in the treatment of human leukemia. Rubidomycin is an antibiotic of the anthracycline group and is an effective inhibitor of both DNA and RNA synthesis (11).

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Material and Methods. Adult male DBA/2 mice 8- to 12-weeks-old were used. PWM (GIBCO) and PHA (purified phytohemagglutinin, MR 68, Burroughs Wellcome) were dissolved in sterile water. BCG was kindly supplied by Statens Seruminstitut in ampoules which contained 6×10^6 attenuated tubercle baccilli, freeze-dried (3.75 mg tuberculin). Before use the content of each ampoule was dissolved to a volume of 5 ml.

The agents were administered ip and rubidomycin (Cerubidin, Scandia Rhodia) was given im in various dosages and time schedules as described in results. The control animals which received rubidomycin only were injected with saline ip. Each group of mice in the survival studies consisted of 20–30 animals. In other groups (three in each) the hemoglobin concentration, leukocyte count, and spleen weight were determined before and with intervals during 15 days after the administration of the various agents.

Results. PWM study. Table I presents the percentage of nonsurvivors of mice treated with either PWM, rubidomycin or both agents. It is seen that PWM in a dose of 10 mg/kg body weight markedly increased the toxic effect of rubidomycin. Thus only 37% of mice treated with 10 mg PWM and 20 mg of rubidomycin per kg body weight survived for a month, whereas 95% of mice treated with rubidomycin alone survived. This difference is statistically significant ($p < 0.0001$). However, PWM was unable to increase the toxicity of rubidomycin to such an extent that rubidomycin in a dose of 10 mg/kg body weight could be lethal to mice. Likewise it is seen from Table I that PWM in a dose of 1 mg/kg body weight was not

TABLE I. Mortality of DBA/2 Mice After Treatment with Rubidomycin and PWM.

Dose of PWM per kg body weight (mg)	Dose of rubidomycin per kg body weight (mg)	No. of mice	Per cent mortality at 30 days	p^a	Mean survival time of nonsurvivors (days)	p^b
10	0	20	0	—		—
0	10	20	0	—		—
10	10	20	0	—		—
0	20	112	5.4	—	19.5	—
10	20	30	63.3	<0.0001	18.3	>0.05
1	20	20	10.0	>0.05	20.5	—
10 (day +6)	20 (day 0)	20	5.0	>0.05	27.0	—
10 (day +3)	20 (day 0)	20	20.0	>0.05	26.8	—
10 (day −6)	20 (day 0)	20	10.0	>0.05	24.0	—
10 (day −3)	20 (day 0)	20	10.0	>0.05	21.5	—

[a] p test for mortality: 2 × 2 test.

[b] p test for mean survival time: non-parametric Man Whitney Ranksum test.

able to intensify the action of 20 mg rubidomycin/kg body weight significantly. From Table I it is also seen that in mice treated with PWM either 6 days before or 6 days after rubidomycin, no increase in mortality was seen as compared to mice treated with rubidomycin alone. In mice which received PWM 3 days before or 3 days after rubidomycin only a slight increased mortality was seen, which was not significant.

Figure 1 shows that rubidomycin in a dose of 20 mg/kg body weight produced a leukopenia (0.76×10^6 leukocytes/ml—a reduction by 71%) followed by an elevated leukocyte count after 10 days. Similarly, the spleen weight/body weight ratio initially decreased. About 10 days after the administration of rubidomycin an increase in this ratio occurred. Similar findings were made when PWM was given together with rubidomycin.

PHA study. Table II shows that PHA in a dose of 10 mg/kg body weight did not increase the toxic effect of 20 mg rubidomycin/kg body weight, nor was PHA able to protect mice against the lethal effect of rubidomycin given in doses of 30 mg/kg body weight. Figure 1 shows leukocyte counts and spleen weight/body weight ratios in PHA treated animals comparable to those found in mice treated with rubidomycin alone.

BCG study. Table III shows that when BCG was given as both single and repeated injections (with intervals of two weeks) together with rubidomycin a slight increase in mortality was seen as compared to mice which had received rubidomycin only. However, the differences were not statistically significant. As in the case of PHA and PWM very little effect was seen of BCG on leukocyte counts and spleen weight ratios (Fig. 1).

Discussion. Recently, several studies have appeared which suggest that PHA and PWM may exert an effect on the immunological apparatus of rodents and influence the effect of ionizing radiation in mice. However, many of the reports have yielded conflicting results. Thus, several authors have demonstrated a radioprotective effect of PHA in mice (9, 12) and furthermore PHA has been shown to produce an increased number of endogenous, hemapoietic colonies in the spleen of irradiated mice (12, 13). However, others have not been able to confirm the radioprotection of PHA (14, 15). Furthermore, it has been shown that PHA suppresses the rejection of skin allografts in mice (16) and the rejection of renal homotransplants in dogs and is able to increase the immunosuppressive effect of azathioprine (17).

The *in vivo* effect of PWM has not been intensively studied. Lozzio and Comas (18) found that PWM markedly depressed the primary immune response in mice and increased the number of endogenous hemopoietic spleen colonies in irradiated mice. We have not been able to find any studies in the literature dealing with similar *in vivo* effects of BCG. How-

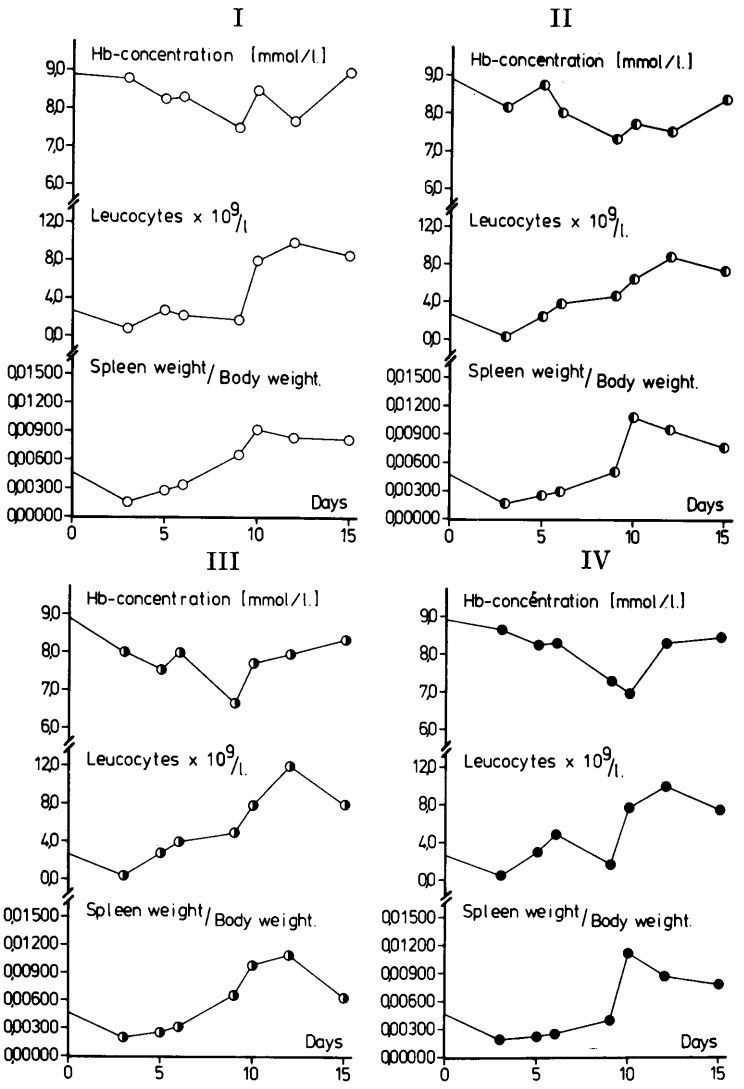

FIG. 1. Hemaglobin concentration, leukocyte count and spleen weight/body weight ratio in mice treated with rubidomycin and BCG, PHA or PWM. (I) 20 mg rubidomycin/kg im + 5 ml saline/kg ip; (II) 20 mg rubidomycin/kg im + 5 ml BCG/kg ip; (III) 20 mg rubidomycin/kg im + 10 mg PHA/kg ip; (IV) 20 mg rubidomycin/kg im + 10 mg PWM/kg ip.

ever, it has recently been demonstrated that BCG is able to produce morphological changes in the lymphoid tissues of mice *in vivo* (5).

In the present series neither PHA nor BCG had any influence on the effect produced by rubidomycin, whereas PWM markedly increased the mortality of mice treated with near-lethal doses of rubidomycin. These results are surprising as the increased toxicity after treatment with PWM and rubidomycin seems to be inconsistent with the aforementioned radioprotective effect of the phytomitogens.

The mechanisms through which PWM increases the toxic effect of rubidomycin are unknown. PWM is nontoxic to mice when given alone in doses used in the present study. One possibility might be that PWM, like rubidomycin, has a cytostatic or cytocidal effect on the bone marrow cells and lymphoid tissue, and thus the increased mortality in mice treated with both agents should be due to a simple synergistic effect. How-

TABLE II. Mortality of DBA/2 Mice After Treatment with Rubidomycin and PHA.

Dose of PHA per kg body weight (mg)	Dose of rubidomycin per kg body weight (mg)	No. of mice	Per cent mortality at 30 days	p^a	Mean survival time of nonsurvivors (days)
0	20	112	5.4	—	19.5
10	20	30	6.7	>0.05	19.5
0	30	20	95.0	—	11.6
10	30	20	95.0	—	11.0

[a] p test for mortality: 2 × 2 test.

ever, when PWM was given to mice treated with rubidomycin, it did not produce further changes in leukocyte count and spleen weight than seen in mice treated with rubidomycin alone. At the same time it may be mentioned that PWM in itself does not reduce the leukocyte count and significantly increases the spleen weight/body weight ratio (5).

Another possibility might be that lymphocytes in the lymph nodes and spleen which may have been transformed by PWM are more susceptible to the toxic effect of rubidomycin than untransformed lymphocytes. However, the results of the experiments with the mice which received rubidomycin 3 and 6 days before or after the administration of PWM seem to exclude this possibility. The finding that PHA produces similar morphological changes in the lymphoid tissues of mice as PWM (5) also speaks against the hypothesis that transformed lymphocytes are more susceptible to the toxic effect of rubidomycin than untransformed lymphocytes. In this connection it may be mentioned that some evidence has recently been presented that PHA and PWM have a different effect on murine lymphocytes, PHA acting on T-lymphocytes, whereas PWM exerts an effect on B-lymphocytes only (19, 20). Possibly this finding may explain why the two phytomitogens have different effects on mice treated with rubidomycin in the present series.

Further *in vivo* studies with PWM and PHA in rodents are clearly needed in order to clarify their effects and modes of action. However, the present study indicates that the combination of rubidomycin treatment with PWM is not advisable. PHA did not increase the toxicity of rubidomycin, but it did not seem to exert any protection against the toxic side effects either. Thus, the present study failed to yield any rational basis for the combined treatment of neoplastic disorders with rubidomycin and phytomitogens. On the other hand, the present experiments did not reveal any contraindications for the combined treatment with rubidomycin and nonspecific immunotherapy.

Summary. The effect of PWM, PHA, and BCG was studied in mice treated with ru-

TABLE III. Mortality of DBA/2 Mice After Treatment with Rubidomycin and BCG.

Dose of BCG per kg body weight (ml)	Dose of rubidomycin per kg body weight (mg)	No. of mice	Per cent mortality at 30 days	p^a	Mean survival time of nonsurvivors (days)	p^b
5	0	20	0	—		
0	20	112	5.4	—	19.5	—
5	20	30	16.7	>0.05	19.4	>0.05
5 × 2 (at day −14 and day 0)	20 (day 0)	32	12.5	>0.05	13.3	>0.05

[a] p test for mortality: 2 × 2 test.

[b] p test for mean survival time: non-parametric Mann Whitney Ranksum test.

bidomycin. PWM markedly increased the mortality of mice which received near-lethal doses of rubidomycin, whereas PHA and BCG did not increase the toxic effect of the cytostatic agent significantly. Neither was PHA able to protect mice treated with lethal doses of rubidomycin. The ip administration of PWM, PHA, or BCG alone produced no systemic toxic reaction in the doses given.

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