

Vitamin Deficiency and Survival of Hepatoma BW7756 Mice

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Introduction¹

Neoplastic tissue in general is a rapidly growing tissue and has substantial requirements for metabolic energy and materials to maintain its integrity. Tumors are reported to have lower levels of vitamins and enzymes than normal tissues and it has been suggested that these deficiencies may offer an approach to tumor chemotherapy (1). Pyridoxine and riboflavin have been shown to be present in low concentrations in certain neoplasms and their metabolic antagonists, desoxypyridoxine and U-2113, a riboflavin antagonists, have been demonstrated to inhibit growth of the 755 mouse tumor (1, 2).

The present investigation was undertaken to extend the studies of inhibition of tumor growth by pyridoxine and riboflavin deficiencies, mentioned above, to BW7756 Hepatoma and to superimpose thiamine and folic acid deficiencies. Findings obtained in this study demonstrate inhibition of this hepatoma by prolonging the survival time of mice bearing this tumor from 110 to 208 per cent of that of control mice on a complete diet.

Materials and Methods

Animals: Male C57L/J mice 3 to 4 weeks old were obtained from the Roscoe B. Jackson Memorial Laboratory, Bar Harbor, Maine. Mice bearing the BW7756 tumor were also obtained from the same laboratory. Tumor fragments taken from these mice were used for implantation into the experimental animals.

Chemicals: Desoxypyridoxine hydrochloride was obtained from Nutritional Biochemical Corp., Cleveland, Ohio; isoriboflavin hydrochloride and oxythiamine chloride-hydrochloride were purchased from Calbiochem Corp., Los Angeles, California. Methotrexate (Lederle) was obtained through the Medical Center Pharmacy. The pyridoxine deficient diet was prepared by General Biochemical, Chagrin Falls, Ohio. The complete diet employed was Wayne Lab-Blox.

Tumor fragments were removed from pentobarbital anesthetized tumor bearing mice and immediately implanted subcutaneously in ether anesthetized mice. The statistical significance of the results were analyzed by the method of Fisher (3).

Results

I. Tumors grown 14 days in mice on complete diet: Tumor transplants were permitted to grow in the mice kept on the complete diet for 14 days at which time the tumor was palpable. The mice were randomly divided into four groups, a control group of six mice,

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and three test groups of thirteen mice each. The control mice were maintained on the complete diet and the test groups were fed the pyridoxine deficient diet. These test groups are given either oxythiamine 100 mg per kg three times a day, isoriboflavin 100 mg per kg two times a day, or desoxypyridoxine 37 mg per kg three times a day. The antagonists were dissolved or suspended in water and injected subcutaneously in the center area of the back. The doses were based on the weight of the mouse taken immediately before administration of the drug.

The antagonists were injected regularly until the mouse became so deficient that it would not walk away when gently pushed. When this state was reached, the mouse was placed in another cage and provided with the complete diet until it had sufficiently recovered to move away promptly when gently pushed. This procedure was repeated until the mouse died. The mouse and its tumor were weighed. Cannibalistic tendencies were noted in all groups of mice. The tumor was frequently cannibalized.

Mean survival time was measured from the start of treatment with antagonist and was $16.3 \pm$ a standard error of 0.07 days for the control group which was kept on the complete diet and had received no antagonist. The mean survival time and standard error of the mean of the test groups were: oxythiamine treated, 33.2 ± 1.7 ; the isoriboflavin treated, 27.2 ± 2.8 ; and the desoxypyridoxine treated mice, 29.8 ± 1.8 days. The null hypothesis was rejected since the probability of such results being obtained by chance was less than one per cent in each of the three experiments (see Table 1).

II. Tumors grown 18 days in mice on complete diet: The tumor transplant was permitted to grow 18 days in mice on a complete diet and at this time the mice were randomly separated into four groups. The control group was given the complete diet until they died. The other three groups were also continued on the complete diet but were treated with vitamin antagonists. The vitamin antagonists were dissolved or suspended in one per cent carboxymethylcellulose and injected into tumor area in a volume of 0.1 to 0.2 ml once daily. These test groups were given a combination of: desoxypyridoxine, isoriboflavin and oxythiamine; desoxypyridoxine, oxythiamine and methotrexate; or desoxypyridoxine, isoriboflavin and methotrexate.

The mean survival time of the control group was 14.3 ± 1.2 days, and the mean survival times of the three test groups were 20.9 ± 0.8 days, 29.9 ± 2.3 days; and 20.9 ± 2.0 days, respectively. The first two test groups means were significantly different from that of the control experiment ($P < 0.1$ in both experiments). The mean of the last group was not significantly different from that of the control experiment ($P < 0.10$). The data are summarized in Table 1.

III. Tumors grown 21 days in mice on complete diet: After tumors had developed in mice on the complete diet, the animals were randomly divided into a control group and three test groups. The control group and two of the test groups were maintained on the complete diet. The first test group was placed on the pyridoxine deficient diet and

TABLE 1
Vitamin Deficiency and Survival of Hepatoma BW 7756 Mice

Mice	Diet	Antagonists	Dose Mg/kg	Survival Days ¹ Mean Range	Per Cent of Control	Statistical Std. Error	Significance Prob- ability
I. Fourteen Day Tumors ²							
6	Complete	None	—	16-17	100	0.07	—
10	Pyridoxine def.	Oxythiamine	100	33.2 14-41	203	1.7	0.01
9	Pyridoxine def.	Isoriboflavin	100	27.2 15-34	167	2.8	0.01
13	Pyridoxine def.	Desoxypyridoxine	37	29.8 16-37	182	1.8	0.01
II. Eighteen Day Tumor Mice ³							
3	Complete	None	—	14.3 12-16	100	1.2	—
8	Complete	Desoxypyridoxine Isoriboflavin Oxythiamine	100 100 200	20.9 16-23	128	0.8	0.01
8	Complete	Desoxypyridoxine Oxythiamine Methotrexate	100 200 0.4	29.9 22-40	208	2.3	0.01
9	Complete	Desoxypyridoxine Isoriboflavin Methotrexate	37 100 0.4	20.9 12-31	146	2.0	0.01
III. Twenty-one Day Tumor Mice ⁴							
6	Complete	None	—	12.7 11-17	100	0.9	—
9	Pyridoxine def.	Desoxypyridoxine Oxythiamine	37 100	16.3 11-29	128	2.5	N.S.
9	Complete	Desoxypyridoxine Oxythiamine Methotrexate	37 100 0.4	17.3 12-25	136	1.7	0.01
7	Complete	Desoxypyridoxine Isoriboflavin Methotrexate	100 50 0.2	14 9-23	110	1.7	N.S.

¹Days starting with administration of antagonists.

²Antagonists injected subcutaneously.

³Antagonists injected into tumor.

⁴Antagonists injected subcutaneously at tumor.

treated with desoxypyridoxine and oxythiamine; the second group was given desoxypyridoxine, oxythiamine and methotrexate; and the third group was treated with desoxypyridoxine, isoriboflavin and methotrexate once a day. The administration of antagonists was continued until the loss of activity as described above occurred. The administration of antagonists was then discontinued until recovery as described earlier. This regimen was repeated until death of the mice.

The mean survival time for the mice fed the complete diet and treated with desoxypyridoxine, oxythiamine and methotrexate (the second test group) had a significantly prolonged life, 17.3 ± 1.5 days when compared to the control mice, 12.7 ± 0.9 days ($P < 0.01$). Mean survival times of the other two experiments were not significantly different from that of the control experiments.

The weights of eleven tumors taken from the control experiment mice varied from 2.3 to 7.1 g and had a mean value of 4.6 g. Intact tumors were recovered from 71 of the vitamin deficient mice. Their weights varied from 1.9 to 7.6 g.

Seven of the vitamin deficient mice apparently died from overdose of vitamin antagonist or accident. Three of the deaths were in the first group and four were in the second group in section I of Table 1. These conclusions are based on the fact that these animals appeared to be in good condition the day before they were found dead and all these mice had small tumors. The largest tumor in these animals weighed 1.9 g. The other six tumors weighed less than 1.5 g. More significant prolongation of survival time was seen in the mice in which vitamin antagonism was started earlier (sections I and II vs. Section III of Table 1).

Summary and Conclusions

Combinations of vitamin deficiencies resulting from a pyridoxine deficient diet and the administration of the vitamin antagonists, desoxypyridoxine, isoriboflavin and oxythiamine significantly prolonged the life of hepatoma BW7756 mice and inhibited the growth of this tumor. These findings are in agreement with those of Shapiro et al. (1, 4) who found similar vitamin deficiencies inhibited 755 mouse tumor growth. It is concluded that deficiencies of pyridoxine in combinations of riboflavin and/or thiamine significantly inhibit the growth of BW7756 mouse tumor and that further study in this area is indicated.

Literature Cited

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