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THE EFFECT OF SELECTED VITAMIN B_{12} ANTAGONISTS AND OTHER COMPOUNDS ON THE C_{1300} MOUSE TUMOR*

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Abstract—Seventeen compounds, including eight competitive vitamin B_{12} antagonists, were tested on the C_{1300} mouse tumor. Marked inhibition was produced by 9-chloroethyl-adenine, and complete inhibition by 6-mercaptopurine. Other compounds were without significant effect. The effect of 6-mercaptopurine was probably unrelated to its anti-B_{12} or anti-purine action.

THE nutritional dependence of animal cells on vitamin B_{12} is well established.[1] Woolley has, however, claimed that the tissues of spontaneous mammary adenocarcinoma in mice are unique, in contrast to non-neoplastic tissues, in their ability to synthesize vitamin B_{12}.[2] Further, Woolley has shown that analogues of 1:2-dimethyl-4:5-diaminobenzene, a precursor of vitamin B_{12}, produce partial regression of a spontaneous mammary adenocarcinoma in the mouse.[3, 4] Regression in a transplanted mouse mammary adenocarcinoma also has been produced by the competitive B_{12} antagonist, 2-ethyl-2:3-naphthimidazole-4:9-dione.[5, 6] In addition, evidence has been obtained for the selective uptake of radioactive B_{12} by the Walker rat carcinosarcoma, and a methylcholanthrene induced hamster sarcoma.[7] These considerations suggested that it would be of interest to test the effect of some recently described vitamin B_{12} antagonists and related compounds[8–11] on a solid tumor system. The C_{1300} mouse tumor, selected for these studies, is at present an undifferentiated round cell tumor, and is reputed to be a neuroblastoma of adrenal origin.[12] *Inter alia*, it resembles the spontaneous mammary adenocarcinoma in the mouse in its general refractoriness to the majority of tumor-inhibiting compounds.[13–15]

METHODS

The effects of seventeen compounds were determined on the C_{1300} tumor system. Approximately half of these compounds had been shown previously to be competitive vitamin B_{12} antagonists in *Euglena gracilis*[9, 11] and in other B_{12}-dependent systems[16–18] (Table 1).

Male CAF_1/JAX mice of 16–21 g weight, and 5–6 weeks of age were used in these experiments. Using a trocar and cannula, uniform tumor fragments approximately

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TABLE 1. INHIBITORY ACTIVITY ON SELECTED VITAMIN B_{12} ANTAGONISTS AND OTHER COMPOUNDS ON THE C_{1300} MOUSE TUMOR

Structure type	Formula	Competitive B_{12} antagonism	Dosage (mg.kg./day)	Tumor inhibition %
(a) Synthetic vitamin B_{12} analogues	Benziminazole analogue	N.T.	50	0–25
	5:6-dichlorobenziminazole analogue	N.T.	50	0–25
	5-methoxybenziminazole analogue	N.T.	50	0–25
	Ethylamide analogue	+ [16, 17]	50	0–25
(b) Benziminazole	1-methyl-2-chloromethyl-6-chloro-benziminazole	− [8, 9]	50	25–50
(c) Nicotinamide derivative	N-2-chloroethyl-β-naphthylamino-ethyl-3-carbonamidopyridinium chloride	+ [9, 11]	5	0–25
(d) Pteridines	4-mercaptopteridine	+ [9,11]	100	0–25
	4-methylmercaptopteridine	+ [9]	50	0–25
	1:3-dimethyl-7-methylmercapto-2:4-dioxo-1:2:3:4-tetrahydro-6-azapteridine	+ [9]	200	25–50
(e) Purines	6-mercaptopurine	+ [9, 11, 18]	25	75–100
	9-furfuryl-8-azaadenine	+ [11]	100	0–25
	2-diethylamino-6-p-dimethylamino-phenylethylpurine	− [11]	200	*
	9-chloroethyladenine	− [9]	100	50–75
(f) Pyrimidines	1:3-dimethylalloxan-5-β-methylthio-carbonylhydrazone	− [9]	25	25–50
	N-(S-methylisothioureido)-1:3-dimethyl-alloxan-5-imide	− [9]	50	0–25
	N-(S-methyl-N-methylisothioureido)-1, 3-dimethylalloxan-5-imide	− [9]	50	0–25
	N-(S-ethyl-N-methylisothioureido)-1, 3-dimethylalloxan-5-imide	− [9]	50	0–25

N.T. — Not tested
* — Marked tumor enhancing effect.

5 mg wet weight were subcutaneously implanted in the right flank. Implant material was obtained from donor animals bearing 12-day-old C_{1300} tumors. Groups of ten mice each were injected intraperitoneally with 0·1 ml suspensions of compounds in sterile Arachis oil. Injections were given daily for 11 successive days, commencing 1 day after tumor implantation, dosage of compounds being determined by previous toxicity tests. In the case of the synthetic B_{12} analogues, toxicity tests could not be performed due to the limited availability of these compounds, and dosage was therefore arbitrary. Control groups were injected with diluent only. All animals were sacrificed on the twelfth day after implantation and the tumors dissected out and weighed.

The effect of massive doses of vitamin B_{12} on the response of the C_{1300} tumor to 6-mercaptopurine was tested in view of recent microbiological evidence that this compound is a competitive B_{12} antagonist.[9, 11, 18] Vitamin B_{12} at 5μg concentration in 0·05 ml of sterile, distilled water was injected subcutaneously and concomitantly with 6-mercaptopurine administered intraperitoneally, as in the above described experiments. Appropriate water, Arachis oil, and vitamin B_{12} control groups were also included in these tests.

RESULTS

One hundred per cent tumor takes were obtained in all experimental groups. No significant weight loss or other obvious evidence of toxicity appeared in any mice under the conditions of test. Only two compounds, 6-mercaptopurine and 9-chloroethyladenine, produced significant inhibtion of tumor growth, the former producing complete inhibition (Table 1). Reversal of the inhibition produced by 6-mercaptopurine was not, however, effected by the simultaneous administration of massive doses of vitamine B_{12}.

One of the compounds, a phenyl-ethyl-substituted purine, produced significant enhancement of tumor growth (Table 1). In repeated experiments, over 100 per cent enhancement, in terms of tumor weights, was consistently observed. No growth-promoting effect, however, was found in another tumor, the Walker carcinosarcoma.[19]

DISCUSSION

6-Mercaptopurine was the only compound producing complete tumor regression. It is difficult to explain the effects of this compound in terms of an anti-purine action alone, as the 6-azapteridine derivative, an equally active anti-purine in microbiological systems[20] (Table 1), produced much less striking inhibition. Another explanation was therefore sought. It is generally assumed that 6-mercaptopurine, one of the most effective drugs for the treatment of acute leukemia, inhibits growth by interfering with nucleic acid and protein synthesis. Natural purines, however, do not reverse its toxic or antineoplastic activity in animals or man,[21] and it was recently speculated that 6-mercaptopurine might interfere with B^{12} metabolism since there is little evidence relating its action in leukemia to an anti-purine effect.[9, 11] Since 6-mercaptopurine, has been shown to produce competitive B_{12} antagonism in several microbiological systems,[11, 18] we had hoped to demonstrate some such effect on the C_{1300} tumor, but the inhibition was not reversed by the concomitant administration of massive concentrations of vitamin B_{12}. The lack of an association between the inhibitory effect of

6-mercaptopurine on the C_{1300} tumor and its vitamin B_{12} antagonism in other systems, is in accord with the resistance of this tumor to other competitive vitamin B_{12} antagonists.

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REFERENCES

1. E. L. SMITH, *Vitamin B_{12}*. Methuen, London (1960).
2. D. W. WOOLLEY, *Proc. Nat. Acad. Sci., Wash.* **41**, 111 (1955).
3. D. W. WOOLLEY, *Cancer Res.* **13**, 327 (1953).
4. D. W. WOOLLEY and G. SCHAFFNER, *Cancer Res.* **14**, 802 (1954).
5. D. MCKENZIE, M. L. STEVENS and R. JONES, *Proc. Amer. Ass. Cancer Res.* **2**, 132 (1956).
6. M. L. ROGERS, D. B. MCNAIR SCOTT, C. ROSE and E. CHU, *American Chemical Society Meeting, Miami* p. 28c (1957) (Abstract).
7. A. MILLER, C. GAULL, H. M. LEMON and J. F. RUSS, *Cancer Res.* **16**, 842 (1956)
8. S. S. EPSTEIN, *Nature, Lond.* **143** (1960).
9. S. S. EPSTEIN and G. M. TIMMIS, *J. Protozool.* In press.
10. E. L. SMITH, L. PARKER, D. E. GANT, Vitamin B_{12}. *Biochem. J.* **62**, 14p (1956).
11. G. M. TIMMIS and S. S. EPSTEIN, *Nature, Lond.* **184**, 1383 (1959).
12. L. C. DUNHAM and H. L. STEWART, *J. Nat. Cancer Inst.* **13**, 1299 (1953).
13. B. L. FREEDLANDER and F. A. FRENCH, *Cancer Res.* **18**, 360 (1958).
14. F. A. FRENCH and B. C. FREEDLANDER, *Cancer Res.* **18**, 172 (1958).
15. G. L. WOODSIDE, G. W. KIDDER, V. C. DEWEY and R. E. PARKS, *Cancer Res.* **13**, 289 (1953).
16. H. BAKER, O. FRANK, L. PASHER, S. H. HUTNER and H. SOBOTKA, *Proc. Soc. Exp. Biol., N.Y.* **104**, 33 (1960).
17. J. E. FORD, *Gen. Microbiol.* **21**, 693 (1959).
18. D. PERLMAN, C. W. DELISLE, N. A. GIUFFRE, *Proc. Amer. Ass. Cancer Res.* **3**, 258 (1961).
19 G. M. TIMMIS. Personal communication.
20. P. F. D'ARCY and A. COX. Personal communication.
21. J. H. BURCHENAL, M. L. MURPHY, R. R. ELLISON, M. P. SYKES, T. C. TAN, L. A. LEONE, D. A. KARNOFSKY, L. F. CRAVER, H. W. DARGEON and C. P. RHOADS, *Blood* **8**, 965 (1953).