STUDIES ON THE ROLE OF FOLIC ACID IN THE LEUKEMIC PROCESS

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IT HAS been adequately demonstrated in both human and mouse leukemias that 4-amino-pteroylglutamic acid (aminopterin) and certain derivatives of this folic acid antagonist possess the ability to inhibit preferentially the production of leukemic white blood cells.[3, 6, 12]

Burchenal et al.[4] have recently reported that the antileukemic action of 4-amino-N^{10}-methyl-pteroylglutamic acid can be blocked almost completely by prior administration of ten to twenty times as much folic acid.

The present investigation has involved a study of the effects of large doses of folic acid alone and in combination with aminopterin on the life span of mice with the rather acute transplanted Ak 4 strain leukemia. It appears quite evident that folic acid is a rate-controlling factor in this particular leukemia. An excess of folic acid speeds up the leukemic process, causing the animals to die before untreated controls. Administration of a compound (aminopterin) that antagonizes folic acid increases the life span of leukemic mice significantly, and thirdly, the antileukemic activity of a folic acid antagonist (aminopterin) can be reversed by administration of relatively large amounts of folic acid.

EXPERIMENTAL PROCEDURE

The antileukemic assay procedure employed has been described in a previous publication.[11] Leukemia-susceptible Akm mice were inoculated with Ak 4 leukemia and treatment with aminopterin was begun after two days. Folic acid was injected intraperitoneally, beginning twenty-four hours after inoculation. The injection schedule was uniformly on an alternate-day basis for a total of ten injections, or for as long as the mice survived. The diet employed in these experiments consisted of Purina laboratory chow supplemented with bread and milk and raw carrots.

RESULTS

The results of the three separate experiments with individual control groups are self-consistent. The summarized data are given in Table 1. The internal consistency of the experiments is indicated in the simple statistical analysis. From the statistical point of view, there is less than one chance in a hundred that the observed accelerating effect of folic acid on the leukemic process might be anomalous.

DISCUSSION

Woolley and Pringle have recently noted the formation of 4-amino-5-carboxamido-imidazole during growth of *Escherichia coli* in the presence of 4-amino-pteroylglutamic acid. This possible purine precursor, first observed to accumulate during sulfonamide bacteriostasis by Stetten and Fox and later identified by Shive et al., offers interesting possibilities as a basis for the biochemical explanation of the present results and those of Burchenal.[4] Gordon et al. have suggested that formyl folic acid is a functional derivative of folic acid, perhaps being involved in the introduction of the single carbon unit into purines and pyrimidines. Sonne, Buchanan, and Delluva

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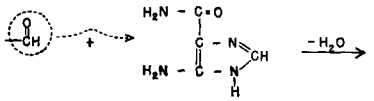
have injected isotopic formate into pigeons and observed that 72 per cent of the ureide carbon of the newly formed uric acid was derived from the administered formate. If, as Woolley suggests, aminopterin leads to a folic acid deficiency, which in turn is responsible for a failure in purine synthesis, then it would appear that the rate-controlling function of folic acid in leukemia might be found in the last step of biosynthesis of the purine skeleton, that step being the transfer of formate via formyl folic acid to the 2-position of the purine skeleton.

Folic acid + HCOOH →

Folic acid (Formyl folic acid) +  → (4-Amino-5-carboxamidoimidazole)

Folic acid + Purine skeleton

(In view of the observation of Getler et al. that free hypoxanthine is not a precursor of nucleic acid purines, it would appear that either some derivative of 4-amino-5-carboxamidoimidazole or hypoxanthine, possibly the ribosides of these compounds, might act as purine precursors.)

N^{10}-Methylpteroylglutamic acid,[5] N^{10}-phenacylpteroylglutamic acid,[5] and 9,N^{10}-dimethylpteroylglutamic acid[8] are three examples of the complete loss of growth-promoting activity of the basic folic acid structure that accompanies blocking of the N^{10} group.

It has been reported by Bethell et al. that the folic acid content of human leukemic leukocytes is much higher than that of normal leukocytes. If the requirement of purines, purine nucleosides, and purine nucleotides (for formation of cellular nucleoproteins) is greater in the more dynamic leukemic cell-forming tissue than in normal cells, then an excess of folic acid might be expected to speed up the leukemic process and a deficiency might preferentially depress malignant mitosis. Such results have been obtained in the present experiments.

This theory is, of course, not original, but if it can be further substantiated (tracer experiments with this objective are under way in this laboratory), a clearer understanding of the biochemical mechanism of action of a known type of antileukemic agent might be forthcoming.

TABLE 1

ANTILEUKEMIC ASSAY DATA

Exper. no.	Dose (mg./Kg.) Folic acid	Dose (mg./Kg.) Aminopterin	No. mice	Survival time (days) Range	Survival time (days) Mean	Survival time (days) S.D.*
1	—	—	10	7–14	8.8	2.2
	60	—	10	7–8	7.2	0.4
	—	0.23	10	14–20	16.8	2.2
	—	0.18	10	14–19	15.7	1.7
	—	0.12	10	10–17	13.9	1.6
	—	0.06	10	8–13	9.8	1.3
	60	0.23	10	13–16	14.0	1.1
2	—	—	10	7–10	8.4	1.2
	60	—	10	7–8	7.5	0.5
	—	0.23	10	12–18	15.7	1.6
3	—	—	10	7–12	10.2	1.6
	60	—	10	8–9	8.1	0.3
	—	0.23	20	10–17	14.0	2.0
	60	0.23	10	8–13	11.0	1.3

* Standard deviation

REFERENCES

1. BETHELL, F. H., and SWENDSEID, M. E.: The folic acid content of leukocytes: Observations on normal subjects and persons with leukemia. *J. Clin. Investigation* 25: 917-918, 1946.

2. BROWN, G. B.: Studies of purine metabolism. *Cold Spring Harbor Sym. Quant. Biol.* 13: 43-51, 1948.

3. BURCHENAL, J. H.; BIEDLER, J. L.; NUTTING, J., and STOBBE, G. D.: The effects of 4-amino-N^{10}-methyl-pteroylglutamic acid on the leukocytes of the normal and leukemic mouse. *Blood* 5: 167-176, 1950.

4. BURCHENAL, J. H.; JOHNSTON, S. F.; BURCHENAL, J. R.; KUSHIDA, M. N.; ROBINSON, E., and STOCK, C. C.: Chemotherapy of leukemia. IV. Effect of folic acid derivatives on transplanted mouse leukemia. *Proc. Soc. Exper. Biol. & Med.* 71: 381-387, 1949.

5. COSULICH, D. B., and SMITH, J. M., JR.: Analogs of pteroylglutamic acid. I. N^{10}-Alkylpteroic acid and derivatives. *J. Am. Chem. Soc.* 70: 1922-1926, 1948.

6. FARBER, S.; DIAMOND, L. K.; MERCER, R. D.; SYLVESTER, R. F., JR., and WOLFF, J. A.: Temporary remission in acute leukemia in children produced by folic acid antagonist, 4-aminopteroylglutamic acid (aminopterin). *New England J. Med.* 238: 787-793, 1948.

7. GETLER, H.; ROLL, P. M.; TINKER, J. F., and BROWN, G. B.: A study of the metabolism of dietary hypoxanthine and xanthine in the rat. *J. Biol. Chem.* 178: 259-264, 1949.

8. GORDON, M.; RAVEL, J. M.; EAKIN, R. E., and SHIVE, W.: Formylfolic acid, a functional derivative of folic acid. *J. Am. Chem. Soc.* 70: 878-879, 1948.

9. HULTQUIST, M. E.; SMITH, J. M., JR.; SEEGER, D. R.; COSULICH, D. B., and KUH, E.: Analogs of pteroylglutamic acid. II. 9-Methylpteroylglutamic acid and derivatives. *J. Am. Chem. Soc.* 71: 619-622, 1949.

10. SHIVE, W.; ACKERMANN, W. W.; GORDON, M.; GETZENDANER, M. E., and EAKIN, R. E.: 5(4)-Amino-4(5)-imidazolecarboxamide, a precursor of purines. *J. Am. Chem. Soc.* 69: 725-726, 1947.

11. SKIPPER, H. E., and BRYAN, C. E.: Carbamates in the chemotherapy of leukemia. III. The relationship between chemical structure and antileukemic action of a series of urethan derivatives. *J. Nat. Cancer Inst.* 9: 391-397, 1949.

12. SKIPPER, H. E.; RISER, W. H., JR., and NOLAN, C. W.: The specificity of 4-Aminopteroylglutamic acid (aminopterin) for leukemic hematopoietic tissue. *Blood* 5: 358-371, 1950.

13. SONNE, J. C.; BUCHANAN, J. M., and DELLUVA, A. M.: Biological precursors of uric acid. I. The rôle of lactate, acetate, and formate in the synthesis of the ureide groups of uric acid. *J. Biol. Chem.* 173: 69-79, 1948.

14. STETTEN, M. R., and FOX, C. L., JR.: An amine formed by bacteria during sulfonamide bacteriostasis. *J. Biol. Chem.* 161: 333-349, 1945.

15. WOOLLEY, D. W., and PRINGLE, R. B.: Formation of 4-amino-5-carboxamidoimidazole during the growth of *Escherichia coli* in the presence of 4-aminopteroylglutamic acid. *J. Am. Chem. Soc.* 72: 634, 1950.