

THE USE OF FOLIC ACID ANTAGONISTS IN THE TREATMENT OF ACUTE AND SUBACUTE LEUKEMIA

A PRELIMINARY STATEMENT

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A RECENT meeting of the New York Society of Hematology held on November 30, 1948, was devoted to a symposium on the treatment of leukemia with aminopterin. It was obvious that definite remissions induced by the drug and not of spontaneous nature were being obtained, although the results of therapy in the hands of various workers differed considerably.

In this issue Farber presents a summary of his results with various folic acid antagonists. Since Farber's observations deal almost wholly with children and our own work has been almost wholly with adults, it was thought that a preliminary statement of our own results with these drugs as reported at the above meeting might be of interest.

Thirty five cases of acute and subacute leukemia including 4 children and thirty-one adults, are or have been under treatment with one or more of the folic acid antagonists since mid-April 1948.*

The folic acid antagonists used were: 4 amino, pteroyl glutamic acid—(aminopterin); 4 amino, N¹⁰ methyl pteroyl glutamic acid (a-methopterin); 4 amino, pteroyl aspartic acid—(amino-an-fol); and 4 amino, 9 methyl pteroyl glutamic acid—(a-ninopterin).

These chemicals, dissolved in sterile normal saline, were injected intramuscularly daily until a toxic or pronounced hematologic reaction occurred, following which the drug was discontinued. The drug was then resumed in a maintenance dose when the toxic reaction had subsided. Aminopterin was given in a dosage of 1-4 mg. daily, a-methopterin, 2-5 mg daily; amino-an-fol 25-75 mg. daily, and a-ninopterin 5-15 mg. daily. Maintenance therapy was given either daily or every other day and either by intramuscular or oral route. Tablets of oral aminopterin were ordinarily used in 1 mg. dosage.

Of the 35 cases of acute and subacute leukemia, 1 has been under treatment for less than four weeks, leaving 34 cases for analysis. Of these, 8 died within one to five days after therapy was instituted. Since death occurred so shortly after institution of drug therapy, these cases should probably be excluded from any statistical analysis of the therapeutic effects of the drug. If this is done, 26 cases of acute and

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* The detailed data obtained in these cases will be presented by Drs. William Dameshek, Milton Freedman and Lester Steinberg at a later date.

subacute leukemia are left for evaluation. Of these cases, 9 have had continued or intermittent remissions for at least two months and up to eight and one-half months (as of January 20, 1949). A remission is deemed present when the patient, (a) feels subjectively improved, (b) shows such objective clinical improvement as regression of lymphadenopathy and hepatosplenomegaly and loss of hemorrhagic tendency, (c) shows hematologic improvement as evidenced by improvement in the red cell count, return of leukocyte counts to relatively normal values, a definite increase in blood platelets, and an improvement in the marrow picture, and (d) shows continuous improvement for at least two months.

The remission rates are, therefore: *Gross results*: 34 cases (8 dying in one to five days), 9 remissions = 26 per cent. *Adjusted results*: 26 cases (excluding those dying in one to five days), 9 remissions = 34 per cent.

In the early stages of the study, crude liver extract was used in the attempt to allay the toxic symptoms but this was soon discarded. Folic acid was also used in one case, but since it caused a quick relapse in the leukemic process, it was discarded after a single trial. Penicillin was given routinely in the presence of marked granulocytopenia and/or fever. Transfusions of blood were used to maintain the red cell count at levels of approximately 2.5 to 3.0 M.

The remissions occurring in the 26 cases cited above were further analyzed with respect to the proliferating cell type involved. This is often very difficult because of the primitiveness of the proliferative process. In the more recently studied cases, a "battery" of studies were carried out to determine this previously rather academic question. This included not only the use of the ordinary Romanowsky stains, but oxidase stains, supravital studies, histochemical staining methods including the use of sudan black and phase microscopy.

Best results with the folic acid antagonists were obtained in the lymphoblastic cases. None of the monocytic cases responded.

	<i>Cases</i>	<i>Remissions</i>
Lymphoblastic.....	10	5
Myeloblastic.....	9	3
Monocytic.....	3	0
Unclassified.....	4	1

The greater specificity of the folic acid antagonists for lymphoblastic proliferations is in line with a more or less marked specificity of certain of the chemotherapeutic agents now in use for certain cell types. Thus, nitrogen mustard appears to be most useful in reticulum cell proliferations including Hodgkin's disease, reticulum cell sarcoma, and reticulosis; urethane in granulocytic proliferation of the chronic myelocytic type and in plasmacytoma (multiple myeloma).

Following the development and then subsidence of the toxic reaction to the drug or following the appearance of a reasonably normal white blood cell count, or under both circumstances, a maintenance dose of the drug was given. In recent months, this was usually given by oral route, in a dosage of 1 mg. daily or every other day. Oral aminopterin has proved to be equally as effective as the parenteral medication, causing as marked therapeutic and toxic effects, mg. for mg., as when given parenterally.

Toxic reactions were the rule with aminopterin administration. These depended in great part on the dose used and were in the nature of ulcerative mucous membrane and tongue lesions, nausea, burning sensation in the upper abdomen and diarrhea; a form of vascular purpura and an apparent aggravation of the bleeding tendency.* The marked reduction in leukocyte count and to lesser extent of the other blood elements might be considered as due to a preferential effect of the chemical on the bone marrow. Other folic acid antagonists, such as *a*-methopterin and amino-an-fol were less toxic than aminopterin but in general of lesser therapeutic value.

The impression was obtained that in order to obtain a remission it was necessary to bring about definite so-called toxic manifestations. The margin of safety between a toxic reaction and death was at times very small.

It is the natural objective of the chemist to produce materials with relatively slight degrees of toxicity while maintaining at least a standard therapeutic effect. Recent observations indicate that such a possibility may be present in one of the methylated aminopterin (9 methyl, 4 amino PGA or *a*-ninopterin). In at least one case given this material, therapeutic effects comparable with those of aminopterin were obtained with only minimal toxic effects.

SUMMARY

In summary, the folic acid antagonists have, in varying degrees, the capacity to induce remissions in about one third of the cases of acute and subacute leukemia, in adults as well as in children, and in both leukemic and leukopenic forms.

Clinical, hematologic and (to lesser extent) marrow remissions, are obtained most commonly in the lymphoblastic types, least often in the monocytic types.

It is possible that folic acid is required by the primitive white cell as a growth factor. The folic acid antagonists, which resemble folic acid so strikingly in chemical structure, may result in cell death by modifying various enzyme systems within the primitive cells.

Both clinical and hematologic observations indicate that the proliferative process is by no means cured with aminopterin treatment. Acute leukemia may be likened to "wildfire" which, although damped by aminopterin, continues to "smolder." This smoldering may suddenly light up again into an active leukemic picture, unless continued maintenance therapy is given. Despite maintenance therapy, there finally comes a point in the leukemic process at which both the leukemia and increasing toxicity to drug make further progress impossible, and the patient dies.

Other growth factors or enzymes are probably of at least as great an importance as PGA in the metabolism of the primitive white cells and when these are discovered and their antagonists synthesized, the therapeutic results in acute leukemia may be of more consistent and durable nature. It should be realized further that chemotherapeutic methods against leukemia and the leukocytic proliferations in general (and, in fact, against all proliferative disease) are at least in their very

* A "toxic" reaction may represent simply one or another aspect of the therapeutic response on the part of cells in various parts of the body to the folic acid antagonist. Tissues differ widely in their response to the chemical, and leukemic tissue may be preferentially affected.

infancy. The results thus far obtained in acute leukemia, although to large extent disappointing, indicate that well-defined remissions can be secured in about a third of the cases. For a disease such as acute leukemia, in which remissions previously were highly unusual and of sporadic nature, this indicates a well-defined therapeutic advance and a need for continued investigation along the same general lines.

Differences in results obtained by various groups of workers are difficult to explain. Several points may nevertheless be considered: some of the workers have given inadequate dosage of drug or have failed to use maintenance therapy; some have given folic acid in conjunction with anti-folic acid therapy; in some cases, a crude folic acid antagonist was used; and it is possible that some cases were not observed as minutely as seems necessary. An important factor, which can be determined only by the study of a large group of cases, is the natural variability of acute leukemia from case to case. We have the impression that our best results are obtained in the relatively subacute cases. The fulminating cases, with rapid onset of bleeding and a quick downhill course, are only slightly affected.