Different Patterns of Remission in Acute Myelocytic Leukemia

A Comparison of the Effects of Methyl-Glyoxal-Bis-Guanylhydrazone and 6-Mercaptopurine

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METHYL-GLYOXAL-BIS-GUANYLHYDRAZONE (methyl GAG) is a new chemotherapeutic agent synthesized and reported in 1958 to have anti-tumor activity against mouse leukemia L1210. It has also been shown to be active against acute myelocytic leukemia in man. This compound has induced complete hematologic remission in 11 of 20 consecutive patients with acute myelocytic leukemia treated at the National Cancer Institute. Of these 11 patients, seven underwent a second course of treatment with methyl GAG after relapse; two of the patients achieved a second complete remission. Preliminary observations of the hematologic material from these patients suggested that the morphologic characteristics of the remissions were unique. Further detailed comparison of data from patients treated with methyl GAG with that of patients treated with 6-mercaptopurine (6-MP) revealed different patterns of hematologic remission in the two groups.

MATERIALS AND METHODS

Twenty consecutive patients were treated with methyl GAG from 1960 through 1962. Material from the 11 patients who obtained complete remission with methyl GAG the first time and the two patients who obtained a second remission was examined. For purposes of comparison, patients who had achieved complete remission with 6-MP therapy were studied. Of 139 patients with acute myelocytic leukemia treated with 6-MP from 1953 through 1961, 14 patients had achieved complete hematologic remission. Thus the two groups were not studied concurrently.

Standard criteria were used for the diagnosis of acute myelocytic leukemia. The ages of the patients ranged from 3 to 55 years for the group receiving methyl GAG and from 6 to 63 years for the 6-MP treated patients. Only two of the patients treated with methyl GAG and four of those treated with 6-MP were under the age of 20.

Methyl GAG was administered by daily intravenous infusion in doses of 150 mg. per square meter of body surface area. 6-MP was administered orally in doses of 2.5-3.0 mg. per Kg. Both drugs were continued until a remission was achieved, except that in some cases the dose was reduced or temporarily discontinued because of toxicity.

Adrenocorticosteroid compounds were used sparingly in both groups of patients and it is unlikely that their use influenced the results of this study in any way. Of the 27 patients, 18 (67 per cent) did not receive steroids. Of the nine patients (33 per cent) who did receive steroids, four patients were demonstrated to be refractory to steroids by the finding of marrow replaced with leukemic cells after more than 28 days of continuous steroid
administration before 6-MP (three patients) or methyl GAG (one patient) was started. Steroids were employed for supportive reasons in the remaining three methyl GAG patients late in the course of treatment (14, 21, 53 days), which was only 5, 9, and 7 days respectively before complete remission marrow was obtained. Of the two remaining 6-MP patients, one received steroids concurrently with 6-MP throughout, and in the other steroids were started on day 22 of a 65-day remission induction period. Data from these patients are similar to the entire group of 6-MP patients.

Bone marrow aspirations were performed at 7- to 14-day intervals in the patients receiving methyl GAG; they were performed somewhat less frequently in the 6-MP treated patients. For each group of patients, weekly median values for the different nucleated cellular elements were obtained by interpolation. Hemoglobin concentration, platelet counts, white blood cell counts, and differential counts were determined at least three times weekly.

**RESULTS**

The rate of disappearance of leukemic cells from the bone marrow is shown for both groups of patients in figure 1. In the group treated with methyl GAG there was a rapid decrease of abnormal cells in the bone marrow: less than 10 per cent of the cells were abnormal 4 weeks after the start of drug therapy. By contrast, with 6-MP treatment, leukemic cells disappeared more slowly from the bone marrow and did not reach 10 per cent of the total marrow cells until 8 weeks from the start of treatment. This difference in the rate of disappearance of abnormal cells from the bone marrow was not mirrored in the peripheral blood, as shown in figure 1. Abnormal cells disappeared from the peripheral blood faster than from the marrow and at essentially the same rate for both drugs. Less than 5 per cent leukemic cells remained after 2 weeks.
Differences were also noted for the rate of return of normal cellular elements to the bone marrow as seen in the two upper lines in figure 2. For the methyl GAG-treated group, 90 per cent of the marrow cells were normal at 4 weeks. By contrast, in the 6-MP-treated cases less than 40 per cent were normal at 4 weeks; 90 per cent of all marrow elements were normal 8 weeks after the start of therapy. Differential counts indicated that normoblasts returned at essentially the same slow rate for both groups as shown by the two lower lines in figure 2, and at 4 weeks comprised only 15 per cent of the total marrow elements for both groups. The difference in the rate of return of normal elements to the bone marrow for the two groups could then be accounted for by the marked early return of granulocytes in the group treated with methyl GAG; 4 weeks from the start of therapy a marked granulocytic hyperplasia was seen in the marrow of these patients.

Associated with or preceding the early return of normal granulocytopenia was the morphologic evidence of apparent maturation of leukemic cells. Within a week or two of the initiation of therapy, the marrow contained many immature myeloid cells with some granulation, comparable to the normal myelocyte stage. Only a few of these cells were frankly leukemic with giant nucleoli or bizarre nuclear indentation, yet there was relative uniformity of these immature cells with absence of later stages of the granulocyte series.

Figure 3b contrasts this appearance with that of the untreated marrow (fig. 3a), and with that of the completely normal granulocytopenia seen later with all stages of maturation present (fig. 3c). This pattern was seen in 9 of the 13 patients responding to methyl GAG. There was no similar development
Fig. 3—Characteristic morphologic appearance of bone marrow in patients with acute myeloid leukemia and in patients with acute non-lymphocytic leukemia. (a) Acute myeloid leukemia: biopsy of bone marrow showing myeloblasts. (b) Acute non-lymphocytic leukemia: biopsy of bone marrow showing lymphoblasts. (c) Acute non-lymphocytic leukemia: biopsy of bone marrow showing lymphocytes.
In the patients treated with 6-MP in whom only pictures comparable to either fig. 3a or 3c were seen.

In keeping with the early return of marrow granulocytes during methyl GAG treatment, there was an early appearance of mature polymorphonuclear cells in the peripheral blood, although the total white count fell rapidly to leukopenic levels with both drugs. The absolute granulocyte count for the methyl GAG group reached 1,500 per cu. mm. at 5 weeks, but more than 10 weeks were required before the granulocyte count reached this level in patients treated with 6-MP. Throughout the period of leukopenia there was always a higher percentage of mature granulocytes in the peripheral blood of the patients in the methyl GAG-treated group, as shown in figure 4. Figure 5 shows the difference for the rate of return of platelets to the peripheral blood for the two groups. In the methyl GAG-treated group the platelet count began to rise in the fourth week of treatment to a median level of 150,000 per cu. mm.; the return of platelets was much more gradual for the patients treated with 6-MP.

Megaloblastoid erythropoiesis was noted in 11 of the 14 patients achieving complete remission with 6-MP. Four of the 14 patients had been exposed to amethopterin in combination with 6-MP for varying periods of time. However, only two of these four patients had megaloblastoid changes whereas nine of the remaining 10 patients receiving 6-MP alone had megaloblastoid erythropoiesis. Giant metamyelocytes and hypersegmented polymorphonuclear leukocytes were not observed. Megaloblastoid erythropoiesis was not seen in any
of the patients treated with methyl GAG nor was megaloblastoid erythropoiesis noted in any of these patients prior to therapy.

In more than half of the patients in both groups, there was an early appearance of normal mature lymphocytes in the bone marrow where none had been present prior to the onset of therapy. This lymphocytosis approximated 20–30 per cent of the total marrow cells and occurred early in the course of treatment. It was not seen in patients who did not respond to therapy, and was not associated exclusively with bone marrow hypoplasia. Only three patients treated with 6-MP and two treated with methyl GAG had bone marrow hypoplasia during the induction of remission, while lymphocytosis occurred in about half the patients. By the time disappearance of abnormal cells and return of normal elements occurred, lymphocytes comprised less than 5 per cent of the total cells.

**DISCUSSION**

The evidence presented emphasizes the more rapid bone marrow recovery from acute myelocytic leukemia in patients responding to treatment with methyl GAG than in those responding to treatment with 6-MP. This was reflected in the peripheral blood with a more rapid return of platelets and mature granulocytes which suggests that methyl GAG had less suppressive activity on normal bone marrow elements and more selective effect on the tumor.

The shorter period of pancytopenia with methyl GAG reduced the time the patient was at risk from the complications of sepsis and hemorrhage, and could have, for this reason, contributed to the patient’s ability to achieve a remission with this drug.

It is possible that the difference in rapidity of response to therapy was
related to relative dosage of the two agents. However, Sullivan, et al.\textsuperscript{7} found that when a high loading dose regimen of 6-MP (6.6 mg/Kg.) was employed against acute leukemia of childhood, there was no significant difference in remission rate or rapidity of response to treatment from that seen with standard dose 6-MP (2.5 mg/Kg.) therapy. Thus manipulation of 6-MP does not appear to alter its degree or speed of activity, and leads us to believe that differences in dosage could not account for the differences presented here.

Methyl GAG is a difficult chemotherapeutic agent to use. It is given intravenously daily and requires hospitalization. It can produce severe toxicity, primarily to the gastrointestinal tract. Thus the prompt recognition of onset of remission was an important aspect of therapy with this compound. The most useful parameter was the presence in bone marrow aspirates of early granulation of immature cells. The marrow at this stage shows replacement by cells comparable to normal myelocytes and progranulocytes. Abnormal leukemic cells, mature granulocytes, and normoblasts were present only in small numbers. Although it was not a normal marrow, this appearance was followed by further development of complete remission and normal marrow in all patients, even if further therapy was not given. Thus this morphologic appearance of the marrow was the hallmark of impending remission with methyl GAG and could be used as an end-point of therapy.

A noteworthy finding was the dissociation of recovery of normal erythropoiesis and granulocytopoiesis in patients on methyl GAG. Erythroid hyperplasia along with reticulocytosis is one of the earliest indications of remission with prednisone, 6-MP, and antifolic agents in the acute leukemia of childhood. This was also true of the 6-MP-treated patients with acute myelocytic leukemia. Leukemic cells may still be present at this stage but recovery of granulocytes was nearly simultaneous with the gradual disappearance of leukemic cells and the appearance of erythroid hyperplasia. In contrast, with methyl GAG, recovery of granulocytopoiesis clearly preceded that of normoblasts. It is possible that the slow return of normoblasts to the bone marrow with both agents represented a suppressive effect. If so, the suppression was about the same for both drugs. It is known that methyl GAG can selectively suppress erythropoiesis as judged by experience with solid tumor patients\textsuperscript{8} and by attempts at maintenance therapy in acute myelocytic leukemia. Erythropoiesis is suppressed by 6-MP as well, though not selectively.\textsuperscript{9}

In the majority of patients achieving remission on 6-MP, erythropoiesis was abnormal. This incidence of megaloblastoid change was higher than previously reported.\textsuperscript{10} The morphologic changes in the red cells were indistinguishable from those occurring in therapy with folic antagonists.\textsuperscript{11} These changes could also be seen in some patients who did not respond to therapy with 6-MP which seems to indicate that the cause of the abnormal erythropoiesis was not necessarily related to the anti-tumor effect of the drug.

It has been shown in the study of rodent leukemia that a tumor resistant to 6-MP will respond to methyl GAG and that a tumor resistant to methyl GAG will respond to 6-MP.\textsuperscript{12} This lack of cross-resistance between the two
drugs in the treatment of rodent leukemia, coupled with the information presented here demonstrating the different patterns and morphologic changes associated with the use of these two agents in human leukemia, would indicate that different mechanisms of action are involved. There is considerable evidence available that the effectiveness of 6-MP can be attributed to inhibition of purine nucleotide biosynthesis. The mechanism of action of methyl GAG is not known.

As 6-MP is known to be myelosuppressive to normal bone marrow, it is possible that the pattern of remission and recovery with 6-MP might be similar to that of methyl GAG if granulocytopoiesis were not suppressed. However, this would not explain the typical morphologic appearance described in the early marrows of patients treated with methyl GAG. The appearance of the relative uniformity of the immature cells with granulation seen in the patients responding to methyl GAG may well represent maturing leukemic cells. This contrasts markedly with the slow disappearance of leukemic cells in patients responding to 6-MP, and their gradual replacement by normal mature granulocytes and normoblasts. The difference in pattern and morphology suggests also that acute myelocytic leukemia is capable of at least two distinct histopathologic pathways of recovery.

**Summary**

Methyl-glyoxal-bis-guanylhydrazone (methyl GAG) has induced complete hematologic remissions in patients with acute myelocytic leukemia. The hematologic pattern of remission was different from that observed for 6-mercaptopurine. Early lymphocytosis and increase in platelet counts occurred with both drugs. With methyl GAG the early granulation of immature cells and a relative rise in peripheral mature granulocytes developed. This could occur with persistent leukopenia and could precede the recovery of platelets. Repopulation of red cell precursors occurred at a similar rate with both drugs. Abnormal megaloblastoid erythropoiesis was seen in 11 of 14 patients treated with 6-MP; erythropoiesis was normoblastic in all patients treated with methyl GAG.

Recognition of the different patterns of remission characteristic of 6-MP and methyl GAG suggests different mechanisms of action for the two drugs. The criteria of impending remission observed with methyl GAG may make possible shortened administration of a toxic drug.
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Le recognition del differente modos de remission que es characteristic de 6-mercaptopurina e de methyl-GAG suggere differente mechanismos de action pro le duo pharmacos. Le criterios de un imminente remission observate con methyl-GAG permitte possibilemente un accurtate administration de iste toxic pharmaco.

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REFERENCES

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