

5-Azacytidine in Childhood Leukemia

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5-Azacytidine was given to 20 children with acute leukemia and to one child with chronic myeloid leukemia. The application of 5-Azacytidine alone during the induction of first remission resulted in 90 % cases in a considerable decrease of leukocyte counts, in the disappearance of blasts from the blood or in their depression and in an improvement of clinical symptoms. After the addition of Prednisone to 5-Azacytidine or after the initial combination of Prednisone with 5-Azacytidine, a complete remission was obtained in 10 out of 12 cases (83.3 %). During more advanced periods of leukemia evolution the combination of 5-Azacytidine with Prednisone caused depletion of leukocytes and blasts in the blood in 75 % of cases without substantial influence on the bone marrow. The tolerance of 5-Azacytidine is good. Pronounced clinical improvement with transient normalization of the blood formula was observed in one child with typical chronic myeloid leukemia.

In recent years numerous analogues which interfere with cellular nucleic acids metabolism have been investigated for their effect upon various malignant neoplasms in both experimental animals and humans.

5-Azacytidine (5-AzCR), a compound closely resembling cytidine in chemical structure (Fig. 1) has been synthesized by PÍSKALA and ŠORM [12]. It is incorporated with cell nucleic acids [11] and it affects RNA synthesis *de novo* by inhibiting orotidylic acid decarboxylase [16]. In mice, its effects are directed primarily against lymphoid tissue and bone marrow causing a considerable depression of RNA and DNA synthesis especially in large lymphocytes and myeloid cells [17]. The drug is capable of provoking interruption of pregnancy in mice [14] and, at nontoxic doses to mothers it causes almost complete destruction of mouse fetuses or their malformations [13]. Its inhibitory effect on the growth of leukemic cells in mice is particularly remarkable [15, 16]. On account of favourable action of 5-Azacytidine against experimental leukemia it seemed desirable to investigate its effect upon hemoblastosis in children.

This report summarizes for the first time the results obtained with 5-Azacytidine in twenty children with acute leukemia, and in one child with chronic myeloid leukemia treated at the 2nd Children Clinic in Prague (16 cases of acute leukemia and 1 case of chronic myeloid leukemia), and at the Department of Pediatrics, Prof. R. GOSTOF, Prague City Hospital, Bulovka (4 cases of acute leukemia in 1967—1970).

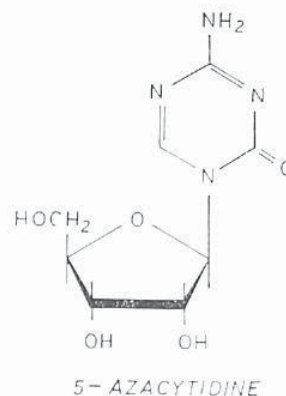


Fig. 1. Structure of 5-azacytidine.

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Table 1. Induction of the first remission with 5-Azacytidine (2nd Children Clinic).
1-5, ALL; 6, AML; 7, Erythromyeloidosis (1.330 erythroblasts per mm)

	Blood		Bone marrow blasts (%)	Therapy	Result				Duration of first remission
	WBC	blasts (%)			WBC	blasts (%)	bone marrow (%)	clinical condition	
1 8y	11 200	78	96	5-AzCR 14 days	1 700	13	not examined complete	improvement and organ shrinkage remission	16 months
2 4y	42 700	59	94	5-AzCR + Predn. 14 days	3 900	—	56 complete	improvement and organ shrinkage remission	6 months
3 3y	26 000	64	96	5-AzCR 1 month 5-AzCR + Predn. 11 days	7 250 300	25 26	92 94	improvement	—
4 3y	10 500	37	99	5-AzCR 2 days Predn. 20 days 5-AzCR + Predn. 14 days	2 000	5	not examined complete	improvement remission	6 months
5 6y	4 200	11	68	5-AzCR 14 days Predn. + 6-MP Predn. + MTX 3 weeks	6 400	29	75 complete	improvement remission	2 months
6 7y	19 000	61	65	5-AzCR 12 days Predn. + Onco + 6-MP 1 month	4 900 1 140	21 1	62 4	improvement remission	3 months
7 3y	10 200	14.5	10 Mybl.	5-AzCR 1 week 5-AzCR + Predn. + 6 MP 3 weeks	4 470 16 500	2.5 38	no improvement no improvement	small improvement 14 days expired-sepsis after 2 months	—

Leukemias were classified on the morphological and cytochemical basis. The group including blast leukemias of nonmyeloid origin (undifferentiated, lymphoblastic and lymphoreticular) with negative Sudan Black reaction and with PAS reaction positive to a different degree was designated as ALL. Leukemias with myeloblasts showing positive Sudan Black reaction and negative granular reaction to glycogen were specified as AML.

For the evaluation of therapeutic response, the internationally accepted criteria for the classification of complete remission in children [2, 7] were employed: A. In the bone marrow the disappearance of morphological aberrations and the decrease of blasts under 5 per cent, lymphocytosis under 20 per cent, normal granulo-, erythro- and thrombocytopoiesis. B. In the blood hemoglobin values higher than 11 Gm. per cent, in children younger than 2 years 10 Gm. per cent, normal number of leukocytes and circulating granulocytes, the thrombocyte count above 100 000 per cmm. C. The disappearance of abnormal physical findings. D. The disappearance of the clinical symptoms of the disease.

First group includes 7 children (Table 1) that received 5-Azacytidine during the initial phase of the disease as the only medication at the dose level of 1-2 mg per kg daily besides transfusions which were sometimes inevitable on account of serious anemia. 5-Azacytidine was administered only in cases with leukocyte

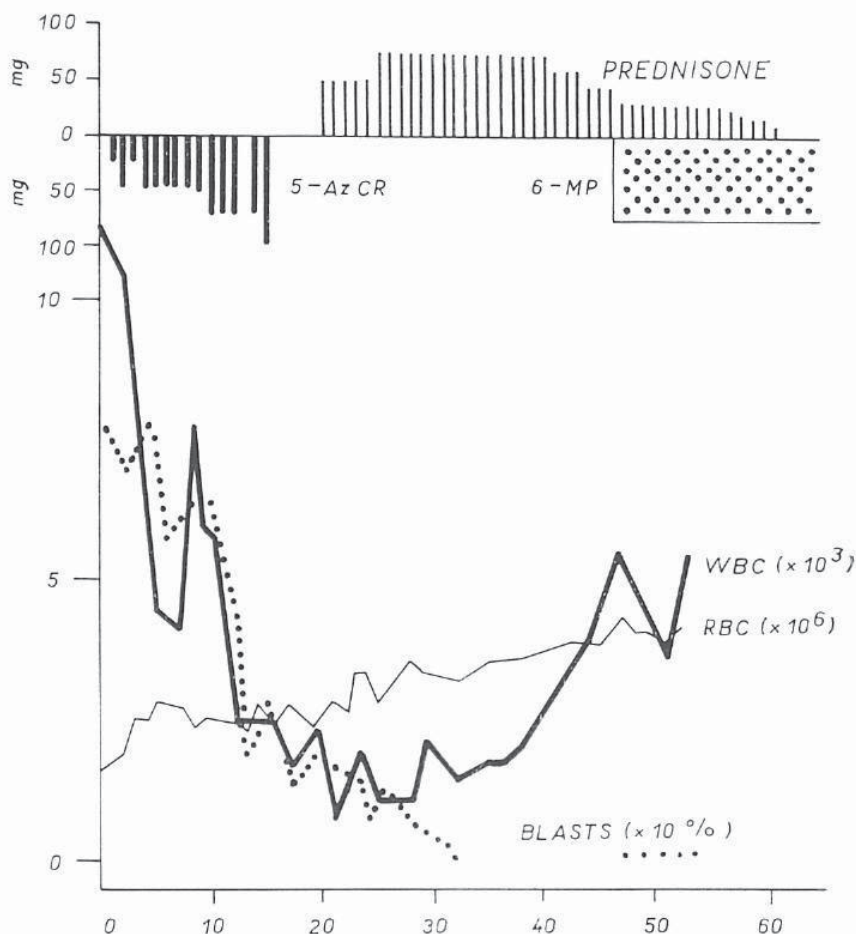


Fig. 2. Hematological evolution during the induction of first remission in patient No. 1, 8 years, ALL.

Table 2. Induction of the first remission with 5-Azacytidine alone and 5-Azacytidine with Prednisolone (Department of Pediatrics). 1-4, ALL

		Blood		Bone marrow blasts %	Therapy	Result			Duration of first remission	Further evolution
		WBC	blasts %			WBC	blasts %	Clinical condition		
1	2 1/2 y	6 200	39	unif.	5-AzCR + 5-AzCR + Predn. 1 week 5 weeks (3 days)	3 100 9 000	86 —	improvement complete remission	6 months	II and III partial remission 5-AzCR + Predn. + 6-MP Predn. + MTX Predn. + Cyclo expired after 1 1/2 year
2	7 1/2 y	239 000	92.5	unif.	5-AzCR + 5-AzCR + Predn. 1 week 2 weeks (2 days) Predn. — 2 weeks	147 000 10 600	82 —	improvement and organ shrinkage complete remission	0.5 month	II, III, IV, V, partial remission Predn. + 6-MP Predn. + 5-AzCR + MTX Predn. + MTX expired after 9 months
3	4 1/2 y	5 600	17	33	5-AzCR 13 × 35 days Predn. 20 days	3 600	—	improvement and organ shrinkage complete remission	3 months	II partial remission Predn. + 5-AzCR 3 months expired after 9 months
4	2 1/2 y	25 300	60	unif.	Predn. 1 week 5-AzCR + Predn. 3 weeks	2 300 4 100	12 —	improvement and organ shrinkage complete remission	?	not followed up

count more than 4000 per cmm and without grave hemorrhagic diathesis which would render the intramuscular application impossible. The therapy was discontinued when 1. the findings in the bone marrow indicated that the treatment did not result in a complete remission after 14 to 30 days, or 2. when the decrease in the leukocyte or thrombocyte count indicated the discontinuance of 5-Azacytidine and corticoid treatment or of the combination of both of these drugs.

The results are summarized in Table 1. It is evident that in all cases with the exception of case number 5 the administration of 5-Azacytidine resulted in a considerable depression of leukocyte counts as well as of blasts in the blood (case No. 1, leukocyte count 11 200 per cmm and 78 per cent blasts to 1700 and 13 per cent; case No. 2, leukocyte count 42 700 per cmm and 59 per cent blasts to 3900 and blast disappearance; case No. 3, leukocyte count 26 000 per cmm and 64 per cent blasts to 7250 and 25 per cent; case No. 4, leukocyte count 10 500 per cmm and 37 per cent blasts to 2000 and 5 per cent; case No. 6, leukocyte count 19 000 per cmm and 61 per cent blasts to 4900 and 21 per cent; case No. 7,

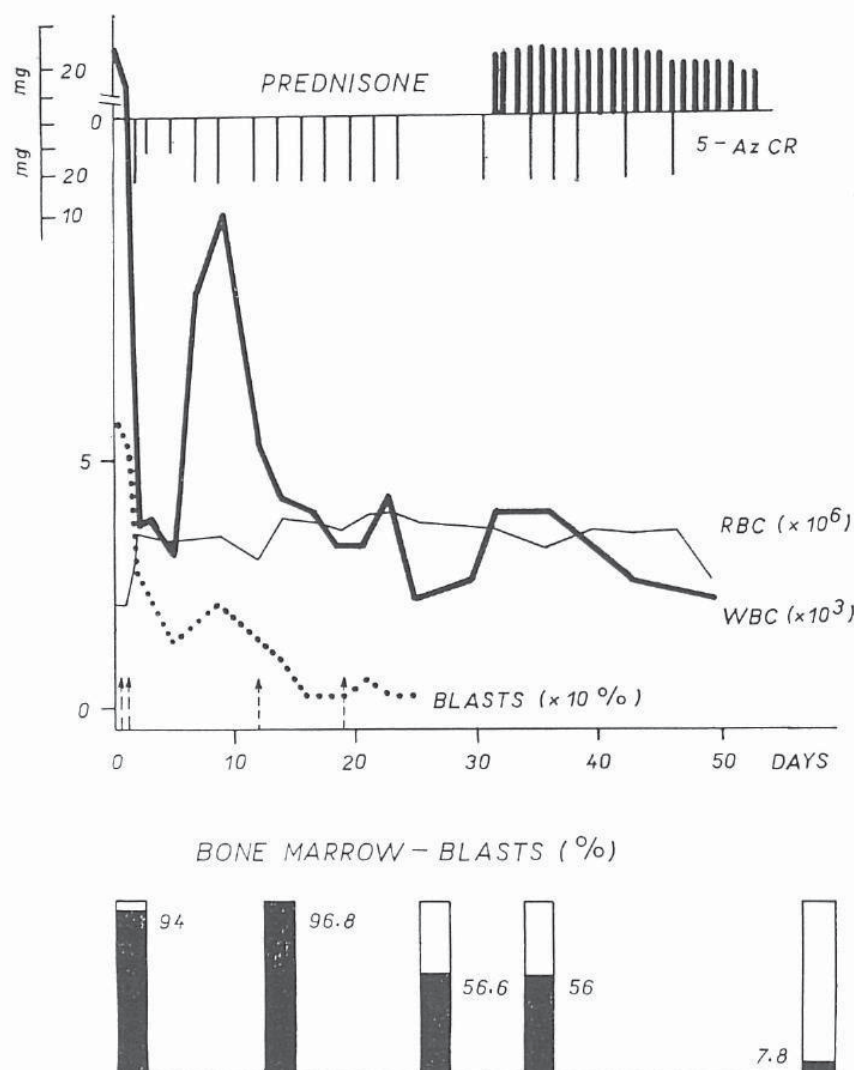


Fig. 3. Hematological evolution during the induction of first remission in patient No. 2, 4 years, ALL.

Table 3. Induction of the first remission with 5-Azacytidine in combination with Prednisone (2nd Children Clinic). 1 — 3, ALL

	Blood		Bone marrow blasts %	Therapy	Result				Duration of first remission
	WBC	blasts %			WBC	blasts %	bone marrow	clinical condition	
1 12y	163 000	98	95.8	Predn. + 5-AzCR Predn. + 6-MP Predn. —	6300 2000 300	16 — —	78.6 78.2 aplasia	improvement improvement expired after 5 1/2 weeks	—
2 9y	51 000	63	92.6	Predn. + 5-AzCR Predn. — Predn. + 6-MP + Onco	1600 3700 2800	9 10 —	not examined 90.6 2.8	improvement complete remission	5 months
3 3y	13 600	33	93.4	Predn. + AzCR Predn. —	1500 4700	3 —	not examined 0.8	improvement complete remission	2 1/2 months

leukocyte count 10 200 per cmm to 4470 and 2.5 per cent blasts.

In 6 out of 7 cases (except case No. 7) the clinical condition distinctly improved; regression of fever and of bone pains could be observed. Shrinkage of infiltrated organs and lymph nodes was considerable in two children (case No. 1 and 2), in remaining patients no significant improvement in this respect was noted. However, in the bone marrow no complete hematological remission has been obtained during the application of 5-Azacytidine alone. In one child the blasts in the bone marrow diminished to 50 per cent (case No. 2, 56 per cent blasts after 1 month of therapy), in two children (case No. 1 and 4) the bone marrow examination was not carried out prior to Prednisone administration; in four patients only diminished cellularity of the bone marrow smears was registered, and the percentage of blasts was not substantially influenced. Following the addition of Prednisone a complete clinical and hematological remission in 5 out of 7 children was obtained (in 4 out of 5 patients with ALL and in 1 out of 2 with AML). These remissions appeared within 4 to 6 weeks after the therapy had been initiated and they lasted 2—16 months (16, 6, 6, 3 and 2 months).

The typical course of changes during the induction of first remission with 5-AzCR is shown in Figs 2,3.

Fig. 2 gives the leukocyte and erythrocyte counts including blasts (per cent) in 8-years old boy suffering of ALL. 5-Azacytidine was given for 2 weeks at a total dosage of 800 mg. The leukocyte count decreased during that period progressively from 11 200 to 1700 per cmm and blasts from 78 to 13 per cent. After another two weeks of therapy upon addition of Prednisone blasts disappeared from the blood and bone marrow and the blood formula returned to normal values. First remission with 6-Mercaptopurine maintenance therapy lasted 16 months.

Fig. 3 illustrates the evolution in 4-years old girl with ALL. The leukocyte count prior to therapy was 42 700 per cmm and 59 per cent blasts. 5-Azacytidine was given over a 1-month period at a total dose of 350 mg. Following first injection (2 mg/kg) the leukocyte count dropped rapidly to 3600 per cmm and blasts diminished to 28 per cent. Following further injections the leukocyte count remained stabilized after 1-month treatment at 3900 per cmm and no blasts were present. In bone marrow smears blasts diminished from 94 to 56 per cent. Complete clinical and hematological remission after further 11 days following Prednisone (2 mg/kg) administration was obtained. First remission lasted 6 months with 6-Mercaptopurine maintenance therapy.

The second group includes 4 children with ALL treated at the Department of Pediatrics, Prague City Hospital, Bulovka in 1967—68 (Tab. 2). In three cases the therapy was initiated with 5-Azacytidine and after 1 week (case No. 1 and 2), or after 5 weeks (case No. 3) combination of 5-Azacytidine with Prednisone was administered. 5-Azacytidine alone promoted a rapid decrease of the leukocyte count (case No. 1 from 6200 to 3100 per cmm, case No. 2 from 239 000 to 147 000 per cmm and case No. 3 from 5600 and 17 per cent blasts to 3600 per cmm and 0). In the remaining case the therapy was initiated with Prednisone during the first week and then the combination 5-Azacytidine and Prednisone was given. Complete clinical and hematological remission was obtained after 6 weeks in case No. 1, 5 weeks (case No. 2), 7 weeks (case No. 3) and after 4 weeks in the remaining case. Bone marrow examination was performed repeatedly only in case No. 1. Later on 5-Azacytidine was given in combination with different drugs while the patients were in relapse. The resulting remissions, however, were only partial.

The third group includes three children with ALL where the first remission was induced with Prednisone in combination with 5-Azacytidine (Table 3). In the first child suffering of peracute leukemia with leukocyte count 163 000 per cmm and 98 per cent blasts in the blood, the combination of both drugs normalized the leukocyte count and depressed blasts to 16 per cent. Clinical condition improved but the amelioration in the bone marrow was only partial. Further therapy with 6-Mercaptopurine in combination with Prednisone resulted in further depletion of leukocytes and blasts in the blood while in the bone marrow their percentage did not change. The disease ended after 4 weeks without remission. The patient developed a serious aplasia and expired.

In the second child with ALL, hematological examination revealed leukocyte count 51 000 per cmm and 63 per cent blasts in the blood. 5-Azacytidine with Prednisone were given with good effect (leukocyte count 1600 and 9 per cent blasts but to obtain a complete remission prolonged treatment with Prednisone and Prednisone-6-Mercaptopurine-Oncovin combination was necessary. The remission lasted 5 months.

In the third child with ALL (leukocyte count 13 000 per cmm and 33 per cent blasts in the blood) the combination therapy resulted within 2 weeks in a rapid fall of leukocyte count to 1500 per cmm and 3 per cent blasts. Complete remission was obtained after further week of treatment with Prednisone alone. First remission lasted 7 weeks.

In the last group including 6 children there were 3 patients with ALL that re-

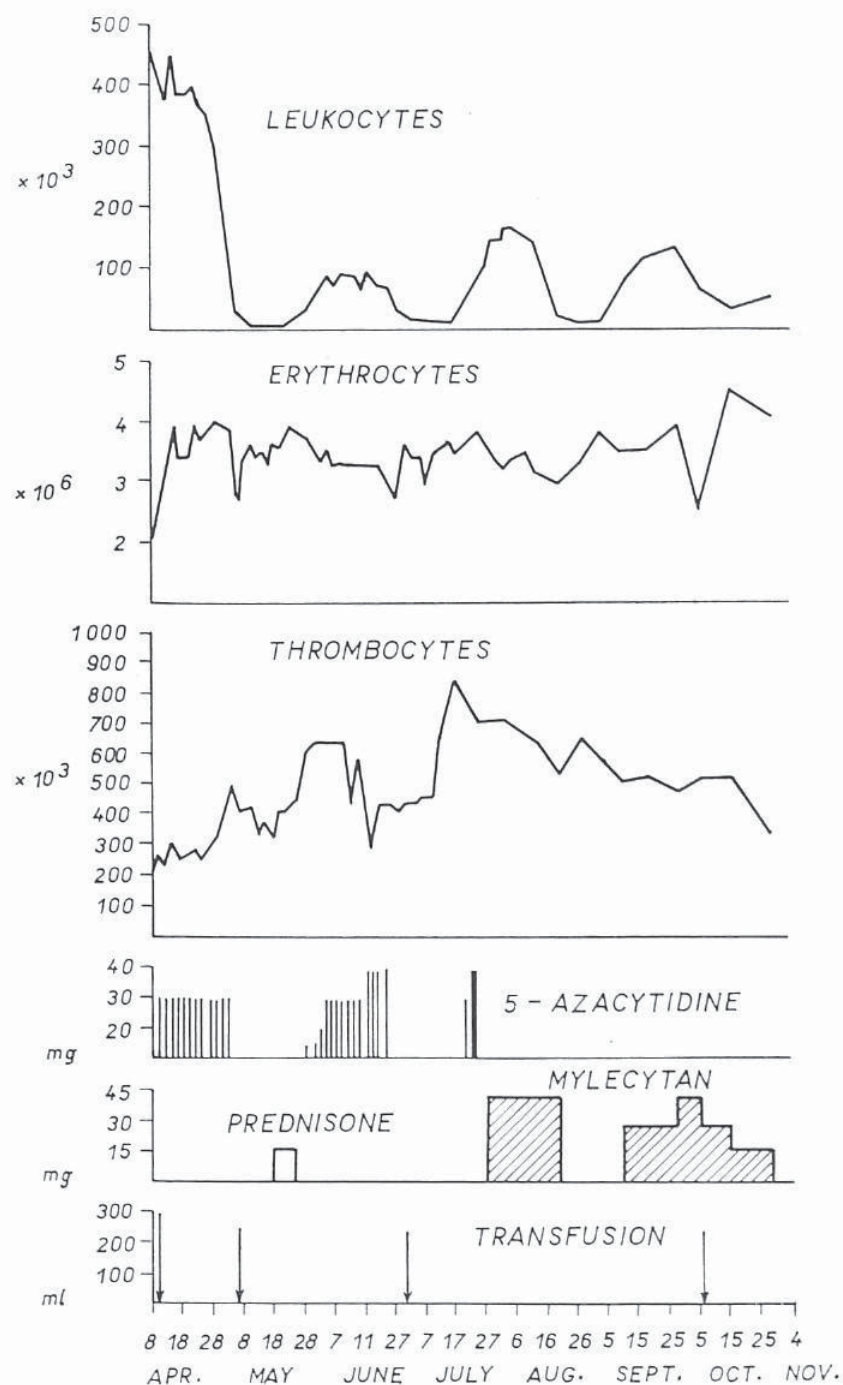


Fig. 4. Hematological evolution during the induction of first remission in 5-years old patient suffering of chronic myeloid leukemia. (Mylecytan 2 mg daily and less.)

ceived 5-Azacytidine or Prednisone in combination with 5-Azacytidine during the first relapse, and 3 cases (2 with ALL and 1 with AML) that were given the same medication during the terminal stage of the disease following the development of resistance to different analogues. The decreased leukocyte count with a considerable drop of blasts in the blood could be registered in 4 out of 6 cases (2 during the first relapse and 2 during the terminal stage); however, bone marrow remained unaffected. In one child during the first relapse that did not react with a complete remission to the combination of Prednisone with 5-Azacytidine such a remission could be obtained with Prednisone and Oncovin.

Of considerable interest is the case of 5-years old boy suffering of typical chronic myelosis. Before admission his parents noted fever, anorexia and enlarged abdomen. Physical examination revealed an enormous splenomegaly; the enlargement of the liver was less considerable while the lymph nodes were not palpable. Hematological examination revealed serious anaemia and hyperleukocytosis (hemoglobin 42 per cent and red blood count 2 millions per cmm; leukocyte count 460 000 per cmm all maturation stages of white cells were present). The number of basophils and eosinophils was elevated, the thrombocyte count was normal. Leukocytic alkaline phosphatases were low (score under 5), fetal hemoglobin 5 per cent. The course of the disease and the response to 5-Azacytidine (1-3 mg/kg daily) is illustrated in the Fig. 4. Within four weeks of treatment with 5-azacytidine (total dosage 760 mg) the leukocyte count dropped to 111 000 per cmm. The therapy was discontinued and further depression to 3600 per cmm with transitory normalization of differential count lasting 3 weeks was observed. The spleen decreased to about half the pre-treatment size and the erythrocyte count improved, while the thrombocyte count remained normal; only one transfusion was given following the admission. Later on, however, the leukocyte count increased to 26 000 per cmm, all maturation forms again appeared including blasts (7 per cent). At this moment, 5-Azacytidine treatment was re-instituted. At first the leukocyte count increased to 97 000 per cmm and then returned to normal values. However, the improvement was not of long duration. Intermittent leukocytosis at that stage was partially controlled with Myleran (0.06 mg/kg daily). The spleen was palpable 3-4 cm below the costal margin. Also the thrombocyte count had an intermittent character.

Conclusions

1. The application of 5-Azacytidine alone during the induction phase of the first remission resulted in 90 % of children in a considerable depression of leukocyte count, decrease or disappearance of blasts in the blood accompanied with the alleviation of clinical symptoms and of abnormal physical findings. The improvement in the bone marrow was either partial or insignificant.

Complete remission was obtained only upon addition of Prednisone generally at the dose level of 1-2 mg/kg daily: In ALL in 10 out of 12 children (83.3 %) and in AML in 1 out of 2 patients. The average duration of remissions was 5.3 months with 6-Mercaptopurine maintenance therapy.

2. The combination therapy with 5-Azacytidine and Prednisone during the induction phase of the first remission had similar effects on the hematological and clinical findings as 5-Azacytidine alone. Leukopenia, however, indicated the discontinuance of 5-Azacytidine application so that ultimately children were treated with Prednisone alone. This group of 3 patients is too small to allow any valid compa-

ri-son with other therapeutic procedures. Equally it is not possible to estimate to what extent 5-Azacytidine accounted for the complete remission in these instances.

3. During the later phases of the disease the effect of 5-Azacytidine or of its combination with Prednisone was generally unsatisfactory. Although the leukocyte count and the depletion of blasts in the blood occurred in 75 % of cases no beneficial effect on the bone marrow was noted. Mild amelioration of clinical condition was only transitory.

4. In one observed case of chronic myeloid leukemia the response to 5-Azacytidine was favourable but of short duration. More cases of chronic myeloid leukemia treated with 5-Azacytidine will be required to verify the first experience.

5. Tolerance of the drug, its toxicity and side-effects: In general the tolerance of 5-Azacytidine is good; in two cases vomiting was observed two to four hours following the intramuscular injection. In the first child vomiting occurred following 14th injection and on re-institution of the therapy at the dose level of 2 mg/kg daily. In the second child vomiting appeared after the 1st and 2nd injection so that the therapy was discontinued. Intramuscular application in children with considerable thrombopenia resulted in hematomas. Other side-effects were not observed.

The effects obtained during the induction of the first remission with 5-Azacytidine alone and later on in combination with Prednisone (83.3 % in ALL) is more favourable than with Prednisone alone (57 % of remissions) or with other drugs given individually [3, 5, 4]. They correspond to the effects of Prednisone in combination with 6-Mercaptopurine (82 %), as well as to the effects of Prednisone with Oncovin according to FREI et al. (84 %) [3], and to our own previous observations [8, 9].

However, they are lower than in case of combination of Prednisone and Oncovin (88 %) [10], in case of combination of Prednisone with high i. v. doses of Methotrexate (93 %), and in case of Prednisone, Oncovin and Rubidomycin combination which is considered at present by different authors as most successful [1, 10] since it induces 90—100 % of remissions. The therapeutic value of 5-Azacytidine requires further investigation especially in combination with other drugs and within the frame of other therapeutic procedures.

The importance of 5-azacytidine for the maintenance therapy has not been further considered on account of its intramuscular application.

Nevertheless, it would be appropriate to examine its action in the short-term reinduction during the remission and in the so called complementary therapy for the remission induction [6]. More cases of CML treated with 5-Azacytidine will be required to test the effect of the drug also in this respect.

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