Efficacy of High-Dose Cyclophosphamide in Combination with Total-Body Irradiation in the Treatment of Acute Myelocytic Leukemia: Studies in a Relevant Rat Model

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ABSTRACT

The efficacy of current clinical leukemia treatment with high-dose cyclophosphamide, supralethal total-body irradiation (TBI), and bone marrow transplantation was evaluated in a rat model for human acute myelocytic leukemia. In rats, both at an early stage of disease and in an advanced stage after remission-induction with 1-β-D-arabinofuranosylcytosine, treatment with cyclophosphamide (100 mg/kg i.p.) followed by either acute, unfractionated TBI (900 centigrays) or fractionated TBI (200 centigrays for seven doses; 450 centigrays for three doses) achieved cure in 90 to 100% and 75% of the animals, respectively. The remaining rats died from treatment-related toxicity despite isologous bone marrow transplantation. Applying the cyclophosphamide-TBI treatment regimens in advanced stage leukemia (tumor load, 5 × 10^9 cells) resulted in death from leukemia relapse in the majority of rats (71%). From the increase in life span after treatment, it was deduced that a 9-log leukemic cell kill was achieved at the most. There was no significant difference between the regimens using fractionated or unfractionated TBI. Toxicity-related deaths occurred mainly in the TBI group receiving 450 centigrays for three doses (38%). In another approach, (repeated) low-dose cyclophosphamide was given subsequent to high-dose cyclophosphamide-TBI treatment applied in advanced stage leukemia. This proved to be effective in eradicating residual leukemia in 80 to 90% of the rats without destroying the bone marrow graft.

In general, the outcome of the various treatment regimens was predictable through accurate information on the tumor load at various stages of disease. The major obstacle in extrapolating the present experimental results to clinical practice is the lack of similar quantitative data in human leukemia.

INTRODUCTION

The treatment of AML by high-dose cyclophosphamide in combination with TBI has become standard treatment in various cancer centers (2, 11, 13). This supralethal treatment modality requires subsequent bone marrow transplantation to overcome irreversible aplasia. If applied in relapse phase, the outcome is generally poor, due to a high relapse rate as well as toxicity-induced deaths (3, 4, 12, 14). Treatment during complete remission appears to be more promising. Various recent studies indicated a projected 3-year leukemia-free survival of 45 to 65% (2, 11, 13). However, apart from graft-versus-host disease and interstitial pneumonitis, leukemia relapse is still one of the major causes of treatment failure. During a recent meeting of the International Society for Experimental Hematology, relapse rates varying from 15 to 45% were reported (10). Thus, despite the lower overall tumor load at the start of therapy in remission, apparently not all leukemia cells are effectively eradicated in a significant number of patients. As neither the number of leukemic cells at the time of treatment is known, nor is information on the efficacy in terms of log leukemic cell kill of the cyclophosphamide-TBI regimen available, the cause of treatment failure remains unclear. Therefore, studies were performed in a transplantable rat leukemia model (BNML) which shares many characteristics with human acute (pro-) myelocytic leukemia, such as a slow growth rate and severe suppression of normal hemopoiesis (6, 9, 16). Such a model offers the advantage of accurate determination of the absolute number of leukemic cells at any given stage of disease, before and after treatment.

In previous publications, the efficacy of high-dose cyclophosphamide treatment and fractionated versus unfractionated supralethal TBI (8) have been reported. This paper describes the quantitative and qualitative effects of cyclophosphamide in combination with TBI applied to various stages of leukemia growth, with particular reference to clinical AML treatment.

MATERIALS AND METHODS

Experimental Animals

The experiments were performed with the inbred BN rat strain produced in the Rijswijk colony. Male rats between 15 and 20 weeks of age were used (body weight, 220 to 290 g).

Rat Leukemia Model (BNML)

The rat leukemia model (BNML) has been described in detail elsewhere (origin, classification, transplantation procedure, growth characteristics, etc., in Refs. 6, 9, and 16). The leukemia was induced in a female BN rat by 9,10-dimethyl-1,2-benanthracene. It shows a reproducible growth pattern upon i.v. cellular transfer within the BN rat strain. Cytologically and cytochemically, it is similar to human acute promyelocytic leukemia. Further analogies with the human disease are: (a) a slow growth rate (10^7 BNML cells killed after 18 to 23 days; growth fraction, 0.60 to 0.40); (b) a severe suppression of normal hemopoiesis due to an absolute numerical decrease in the number of normal hemopoietic stem cells; (c) diffuse intravascular coagulation; (d) prolonged blood transit time of leukemic cells (34 to 36 hr); (e) response to chemotherapy as in human AML; (f) presence of clone-
Cyclophosphamide and TBI in AML

Drugs

Cyclophosphamide (Asta, Weesp, The Netherlands) was dissolved in 0.9% NaCl solution and injected i.p. in a volume of 0.5 ml. ara-C (Upjohn, Ede, The Netherlands) was dissolved in sterile water and injected i.v. in a volume of 0.5 ml.

Total-Body Irradiation

TBI was carried out with a γ-radiation source (Gammacel 220, 137Cs; Atomic Energy of Canada, Ltd.) yielding a dose rate of 115 centigrays/min.

The D₀ for γ-rays of leukemia cells is 85.1 centigrays with an extrapolation number of 3.7 (8).

Experimental Designs

Cyclophosphamide-Fractionated versus Unfractionated TBI at 2 Stages of Leukemia Growth. In a first set of experiments, the efficacy of a single dose of cyclophosphamide followed by either fractionated or unfractionated TBI was studied at 2 stages of disease, i.e., at Days 6 and 13 after inoculation with 10⁷ BNML cells. Cyclophosphamide was given in a dose of 100 mg/kg. This dosage demonstrated the highest therapeutic index in experiments reported previously.³ Three TBI regimens were applied, each starting 24 hr after cyclophosphamide, i.e., 900 centigrays acute, unfractionated (Regimen 1); 450 centigrays daily for 3 doses (3 fractions) (Regimen 3); and 200 centigrays, given twice daily with 8-hr intervals between the daily sessions for a total of 3.5 days (7 fractions) (Regimen 2). Their individual efficacies in terms of tumor load reduction have been described before (8).

Cyclophosphamide-Unfractionated TBI after Remission Induction by ara-C. ara-C treatment (200 mg/kg every 12 hr for 6 doses) was initiated at Day 13 after 10⁷ BNML. This time-sequential chemotherapy regimen based on perturbations of cell cycle kinetics (recruitment-synchronization) is known to induce a complete remission at this advanced stage of disease (1). Two weeks later (Day 27), cyclophosphamide (100 mg/kg) was given followed 24 hr later by 900 centigrays unfractionated TBI.

Cyclophosphamide 'Intensification' after Cyclophosphamide - TBI. Cyclophosphamide (100 mg/kg)-TBI (900 centigrays unfractionated) was applied at Days 13 and 14 after leukemia inoculation. Subsequently, either one or 2 dosages of cyclophosphamide (50 mg/kg each) were given at Day 21 or Days 21 and 27, respectively.

Supportive Care

Transfusion of Blood Cells. In all experiments, 1 ml of packed erythrocytes, leukocytes, and platelets was injected once a day for 4 consecutive days during the interval between high-dose cyclophosphamide-TBI and the stage at which a sufficient number of mature end cells are produced by the transplanted bone marrow (see below). The rapidly induced aplasia could thus be temporarily overcome. In the intensification experiments, 2 additional transfusions of packed cells were given after each low dose of cyclophosphamide. Blood was obtained from normal BN rats through puncture of the abdominal aorta under ether anesthesia.

Bone Marrow Transplantation. Within 24 hr after (the last fraction of) TBI, 10⁷ isologous normal bone marrow cells were injected into a tail vein. This cell dose is more than enough to completely restore normal hemopoiesis (15).

Quantitative Determination of Tumor Load Reduction

The log kill induced by the various treatment regimens was derived from differences in median survival times between treated and nontreated rats, correcting for the duration of treatment (days). Given the linear relationship between the number of inoculated leukemic cells and the survival time, it can be deduced that 1-log cell kill corresponds to an increase in life span of 4 to 5 days (16). Obviously, this requires that the repopulation kinetics of residual leukemic cells in treated rats is similar to the kinetics of proliferation of nontreated controls. Previous studies (7) have indicated that this is the case for tumor loads ranging from 10⁷ to 10⁸ cells.

RESULTS

Cyclophosphamide-Fractionated versus Unfractionated TBI at 2 Stages of Leukemia Growth. In Table 1, the efficacy of cyclophosphamide (100 mg/kg) followed by different TBI regimens applied at an early stage of leukemia growth is demonstrated. All 3 regimens proved to be curative. As the total tumor load prior to treatment is somewhere between 10⁷ and 10⁸ cells, at least a 7- to 8-log cell kill is achieved. With Regimen 3, 2 of 8 rats died from treatment-related toxicity. Toxicity-induced deaths were caused by a combination of hemorrhages in the gastrointestinal tract, the lungs, and the urinary bladder.

Table 1 shows results of the various treatment regimens initiated at an advanced stage of disease, i.e., at Day 13 after inoculation with 10⁷ BNML cells. Only 2 of 24 rats were cured

<table>
<thead>
<tr>
<th>Regimen</th>
<th>Toxicity</th>
<th>Leukemia Cures</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. 100 mg cyclophosphamide/kg i.p. + 900 centigrays acute TBI</td>
<td>0/8</td>
<td>0/8</td>
</tr>
<tr>
<td>2. 100 mg cyclophosphamide/kg i.p. + 200 centigrays TBI 2 times every 24 hr for 3.5 doses</td>
<td>0/8</td>
<td>0/8</td>
</tr>
<tr>
<td>3. 100 mg cyclophosphamide/kg i.p. + 450 centigrays TBI every 24 hr for 3 doses</td>
<td>0/8</td>
<td>0/8</td>
</tr>
</tbody>
</table>

* Treatment was started at Day 6 after inoculation with 10⁷ BNML cells. Each TBI regimen was initiated 24 hr after the injection of cyclophosphamide. The intervals between the daily TBI fractions (Regimen 2) were 8 hr. Within 24 hr after (the last fraction of) TBI, 10⁷ isologous normal bone marrow cells were injected into a tail vein.

Total observation time, >500 days.

Table 2 shows results of the various treatment regimens applied at an advanced stage of disease, i.e., at Day 13 after inoculation with 10⁷ BNML cells. Only 2 of 24 rats were cured

<table>
<thead>
<tr>
<th>Regimen</th>
<th>Toxicity</th>
<th>Leukemia Cures</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. 100 mg cyclophosphamide/kg i.p. + 900 centigrays acute TBI</td>
<td>1/8</td>
<td>7/8</td>
</tr>
<tr>
<td>2. 100 mg cyclophosphamide/kg i.p. + 200 centigrays TBI 2 times every 24 hr for 3.5 doses</td>
<td>1/8</td>
<td>5/8</td>
</tr>
<tr>
<td>3. 100 mg cyclophosphamide/kg i.p. + 450 centigrays TBI every 24 hr for 3 doses</td>
<td>3/8</td>
<td>5/8</td>
</tr>
</tbody>
</table>

* Treatment was started at Day 13 after inoculation with 10⁷ BNML cells. Each TBI regimen was initiated 24 hr after the injection of cyclophosphamide. The intervals between the daily TBI fractions (Regimen 2) were 8 hr. Within 24 hr after (the last fraction of) TBI, 10⁷ isologous normal bone marrow cells were injected into a tail vein.

Total observation time, >500 days.

JANUARY 1983 409

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A. Hagenbeek and A. C. M. Martens

(Regimen 2). The majority of rats died from leukemia relapse (17 of 24), and 5 of 24 rats died from toxicity. Apart from the causes mentioned above, at this stage of disease excessive kill of leukemic cells leading to tumor cell embolism was an additional factor contributing to therapy-related deaths. From the increase in life span of rats dying from relapsed leukemia, the log cell kill was deduced (Table 3). Based on prolongation of the median survival time, all 3 regimens appeared to be equally effective, i.e., an 8- to 9-log leukemic cell kill was achieved. However, a considerable spread of survival times was observed (Table 3, Column 3). A possible explanation for this phenomenon will be discussed later. The expected log cell kill (Table 3, Column 6) was derived as follows. Cyclophosphamide in a dose of 100 mg/kg induces a 5-log cell kill as reported before.3

With the TBI regimens in Experiments 1, 2, and 3, a 4-, 2-, and 3-log leukemic cell kill is achieved, respectively (8). If the combination of both treatment modalities would be purely additive, the expected log cell kill would be 9, 7, and 8 in the respective experiments (Table 3, Column 6). Apart from Experiment 2, the expected and observed log cell kills are quite similar. As the tumor load at Day 13 after leukemia transfer is about 5 x 10⁹ cells (5), the high incidence of leukemia relapse similar. As the tumor load at Day 13 after leukemia transfer is about 5 x 10⁹ cells (5), the high incidence of leukemia relapse was understandable, given the values of additive, the expected log cell kill would be 9, 7, and 8 in the combination of both treatment modalities would be purely additive (Table 3, Column 3). A possible explanation for this phenomenon will be discussed later. The expected log cell kill (Table 3, Column 6) was derived as follows. Cyclophosphamide in a dose of 100 mg/kg induces a 5-log cell kill as reported before.3

Increase in life span by ara-C. From Table 4, it is clear that 100 mg cyclophosphamide per kg followed by 900 centigrays acute, unfractionated TBI given during the phase of complete remission is curative in the majority of rats. Toxicity-induced deaths were observed in 2 of 8 rats. They died 9 and 10 days after the completion of the cyclophosphamide-TBI regimen. A 5.5-log leukemic cell kill was achieved after ara-C alone. Thus, the tumor load at Day 13 was reduced from 5 x 10⁶ to 10⁴ by 200 mg ara-C per kg for 6 doses. Given a doubling time of the leukemic cell population of 1.5 to 2.0 days (7), the total number of leukemic cells present just prior to cyclophosphamide-TBI treatment (Day 27) is about 10⁶. As this regimen induces a 6-to >9-log cell kill (Table 3), its curative potential at this stage of disease could be predicted and was in fact confirmed in this experiment.

Cyclophosphamide Intensification after Cyclophosphamide-TBI. Chart 1 shows the results of either one of 2 low-dose cyclophosphamide intensification treatments following high-dose cyclophosphamide-TBI treatment given at a full-blown stage of disease. After cyclophosphamide-TBI alone, all rats died from relapse leukemia (median survival time, 57 days).

Table 3

<table>
<thead>
<tr>
<th>Regimen</th>
<th>Increase in life span (days)</th>
<th>Log cell kill</th>
<th>Expected log cell kill</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. 100 mg cyclophosphamide/kg i.p. + 900 centigrays acute TBI</td>
<td>33</td>
<td>23-39</td>
<td>8</td>
</tr>
<tr>
<td>2. 100 mg cyclophosphamide/kg i.p. + 200 centigrays every 24 hr for 3.5 doses</td>
<td>36</td>
<td>33-42</td>
<td>9</td>
</tr>
<tr>
<td>3. 100 mg cyclophosphamide/kg i.p. + 450 centigrays every 24 hr for 3 doses</td>
<td>37</td>
<td>9-55</td>
<td>9</td>
</tr>
</tbody>
</table>

- a Treatment was started at Day 13 after inoculation with 10⁷ BNML cells. For further details of the separate regimens, see Table 2, Footnote a. Rats dying from treatment-related toxicity or cured rats are not included in this table.
- b As determined by: (median survival time of treated rats) – (median survival time of nontreated controls) – (duration of treatment). Each group consisted of 5 to 7 rats.
- c MST, median survival time (days).

Table 4

<table>
<thead>
<tr>
<th>Regimen</th>
<th>No. of deaths/no. of rats tested</th>
<th>Increase in life span (days)</th>
<th>Log cell kill</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. ara-C, cyclophosphamide + TBI</td>
<td>2/8</td>
<td>0/8</td>
<td>6/8</td>
</tr>
<tr>
<td>2. ara-C</td>
<td>2/8</td>
<td>6/8</td>
<td>0/8</td>
</tr>
</tbody>
</table>

- a Treatment was started at Day 13 after inoculation with 10⁷ BNML cells. ara-C, 200 mg/kg i.v. every 12 hr for 6 doses; cyclophosphamide, 100 mg/kg i.p. at Day 27; TBI, 900 centigrays acute, unfractionated, at Day 28. At Day 29, 10⁸ isologous normal bone marrow cells were injected into a tail vein (Group 1 only).
- b As determined by: (median survival time of treated rats) – (median survival time of nontreated controls) – (duration of treatment).
- c Total observation time, >500 days.
- d MST, median survival time (days); the 2 rats dying from treatment-related toxicity are not included (Group 2).
Chart 1. Eradication of residual leukemia by (repeated) cyclophosphamide (cyclo) intensification treatment subsequent to high-dose cyclophosphamide and TBI in the BNML. Cyclo (100 mg/kg i.p.), cyclophosphamide (n mg/kg i.p.); TBI, 900 centigrays acute, unfractionated; BMT, bone marrow transplantation, 10° isologous normal bone marrow cells i.v.; MST, median survival time (days); * , 1 ml of packed erythrocytes, leukocytes, and platelets i.v.

In an early stage of leukemia growth, more than 90% of the rats are cured (Table 1). In later stages, i.e., with an increased tumor load, cure is an exception (8%; Table 2). In those situations, treatment-related toxicity (21%) and leukemia relapse (71%) are the causes of death. These findings are quite similar to those obtained with high-dose chemoradiotherapy followed by bone marrow transplantation in the relapse phase of human AML (3, 4, 12, 14). It should be mentioned that, in studies published previously on the efficacy of TBI alone (8), 45% toxicity-related deaths were observed in the 900-centigray unfractionated TBI group. However, in those early studies, no supportive care in terms of repeated transfusions of blood cells after TBI was given. This probably explains the reduced incidence of early deaths in the present experiments. Obviously, graft-versus-host disease does not play a role in these rats because of isologous bone marrow transplantation.

Evaluating cyclophosphamide followed by various TBI regimens, i.e., fractionated versus unfractionated TBI, revealed quite some spread in increased life span (Table 3). It seems unlikely that within one experimental group tumor load reduction would vary from 2 to 9 decades. However, host variation in drug absorption, metabolism, and excretion, even with inbred animals, might have contributed to the differences observed. From the ranges of prolonged survival (Table 3), it is clear that there is almost a complete overlap of the 3 regimens studied. Therefore, no firm recommendation can be given as to the most effective treatment regimen. All 3 seem to have the same potency of leukemic cell kill. Maybe the 3-x 450-centigray TBI regimen (Regimen 3) is slightly more toxic (Tables 1 and 2). The expected log cell kill, based on the assumption that the efficacy of cyclophosphamide-TBI is purely additive, showed a rather good correlation with the actually observed log cell kill with Regimen 2 (cyclophosphamide followed by a 200-centigray dose 7 times), there appeared to be a discrepancy (Table 3). Apparently, this regimen is more effective in terms of log leukemic cell kill than expected. Even 2 of 8 rats were cured (Table 2). The mechanism underlying the synergism in this particular combination is not clear.

DISCUSSION

First of all, from the data presented, it is clear that high-dose cyclophosphamide-TBI is a potentially curative treatment modality. It does not matter whether this regimen is applied to rats not treated previously or to rats after remission induction chemotherapy; the crucial factor is the number of leukemia cells present at the time of initiation of treatment. One of the advantages of studying an animal leukemia model is that the tumor load at any given stage of disease, before or after treatment, can be determined quite accurately. The disadvantages are clear: one animal leukemia model at the most represents only one patient with leukemia. However, from previous studies, it has become clear that the BNML shares many essential characteristics with human AML (6, 9, 16).

In general, up to a 9-log leukemic cell kill could be achieved with various combinations of cyclophosphamide-TBI (Table 3).
If cyclophosphamide-TBI treatment is applied in a stage of disease which is more relevant to the clinical situation, i.e., after remission-induction chemotherapy, the majority of rats are cured (6 of 8, or 75%; Table 4). No leukemia relapses were observed. These results are superior to those obtained in recent clinical studies: 45 to 65% projected 3-year leukemia-free survival; 15 to 45% leukemia relapses (2, 10, 11, 13). This discrepancy might be explained by a smaller tumor load at the time of cyclophosphamide-TBI treatment in the rat (approximately 10^6 leukemic cells) as compared to that in humans. If it is assumed that the cyclophosphamide-TBI regimen used in humans (2 × 60 mg cyclophosphamide per kg followed by 900 to 1000 centigrays TBI) is as efficacious as the optimal combination in the BNML (i.e., a 9-log leukemic cell kill), then this would mean that 15 to 45% of the patients have a leukemic cell burden in excess of 10^6 cells at the time of treatment. This might as well represent the group of patients which would have relapsed early anyway if only maintenance chemotherapy instead of high-dose cyclophosphamide-TBI would have been administered during the phase of complete remission. Theoretically, additional (consolidation) chemotherapy would be desirable in these patients to further decrease the tumor load prior to cyclophosphamide-TBI. However, as long as the detection level of residual leukemic cells remains high with the present conventional means (invisible leukemia below a total tumor load of 10^10 leukemia cells), this particularly high-risk group of patients can not be recognized. Obviously, other factors, including individual differences in pharmacokinetics or drug resistance, might be involved in determining the final outcome of treatment too, i.e., cure or relapse.

Another approach to prevent relapse of leukemia after cyclophosphamide-TBI and bone marrow transplantation is to apply additional chemotherapy after this procedure. In the rat studies, this proved to be very effective even when cyclophosphamide-TBI was applied at an advanced stage of disease (Charts 1 and 2). Single dosages of cyclophosphamide below 50 mg/kg proved to be ineffective, i.e., late relapses occurred in all animals. The crucial question in applying this approach to humans is whether the transplanted regenerated marrow can tolerate additional chemotherapy. It might well be that autologous grafts are more vulnerable to (late) intensification than are allogeneic grafts. So far, no concise clinical data are available at this point. In this respect, the time interval between bone marrow transplantation and intensification chemotherapy as well as the drug dose used is the most critical factor. However, given the unacceptably high incidence of leukemia relapse after bone marrow transplantation in humans, particularly in the autologous and syngeneic situation, it is strongly felt that this approach should be explored in the clinical setting, with the precautions mentioned above in mind.

Finally, the technical impossibility to detect and quantify residual disease in human acute leukemia after both remission-induction chemotherapy and cyclophosphamide-TBI followed by bone marrow transplantation remains the major obstacle in accurately extrapolating the present results obtained in the BNML rat model to humans. Hopefully, new techniques (e.g., using monoclonal antibodies) will make this statement invalid.

ACKNOWLEDGMENTS

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REFERENCES

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