The Use of Long-term Bone Marrow Cultures (LTBMC) to Detect Hematotoxic Side Effects of Purging Methods

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A. Introduction

The development of suitable methods for the purging of malignant bone marrow contaminating cells in patients with acute leukemia may offer a better chance of success for autologous bone marrow transplantation. In addition to investigating the wanted effect, i.e., damage to leukemic cells, it is important to investigate the tolerance of normal hematopoietic stem cells within these manipulations in order to guarantee the grafting of the purged transplants.

Because VP 16-213 is discussed as a potent agent for eliminating tumor cells in vitro [1, 2], we incubated bone marrow with this drug.

Our aims were to determine what doses of VP 16-213 are tolerated by normal hematopoietic stem cells, and whether there is a difference between the behavior of GM-CFC and LTBMC stem cells after drug incubation.

B. Methods

I. Drug Incubation

Bone marrow cells (2×10^7/ml) were incubated for 2 h at 37 °C with different doses of VP 16-213 (50, 75, 100, 125 μM/l), washed twice and cultivated thereafter in the GM-CFC and LTBMC assay.

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II. GM-CFC Assay

GM-CFC were assayed as described elsewhere [3]. The stimulator used was human umbilical cord conditioned medium. Colonies (≥ 50 cells) were counted after 10 days of incubation.

III. LTBMC Assay

LTBMCs were set up according to a modification of the method of Gartner and Kaplan [4]. Briefly, nucleated bone marrow cells (2×10^6/ml) were suspended in IMDM supplemented with 12.5% horse serum, 12.5% fetal calf serum, 10^{-6} M/l hydrocortisone sodium succinate, 10^{-4} M/l mercaptoethanol, 5×10^{-7} M/l sodium selenite, 2×10^{-6} M/l L-glutamine, and antibiotics. The cells were cultivated for 3–5 days at 37° C and thereafter until day 21 at 33° C. The cultures were fed weekly.

Two-stage LTBMCs were established on a 2- to 4-week old preirradiated (15 Gy) adherent layer of normal bone marrow. After 3 weeks the cultures were stopped; the adherent (after trypsinization) and nonadherent cells were united and assayed for GM-CFC.

C. Results

At initiation of all LTBMCs an aliquot of the sample was routinely tested for GM-CFC. The effects of VP 16-213 incubation on GM-CFC are shown in Fig. 1. It is obvious that all doses tested had a strong cytotoxic effect. Considering the mean values of recovery, the cytotoxic effect was more pronounced in bone

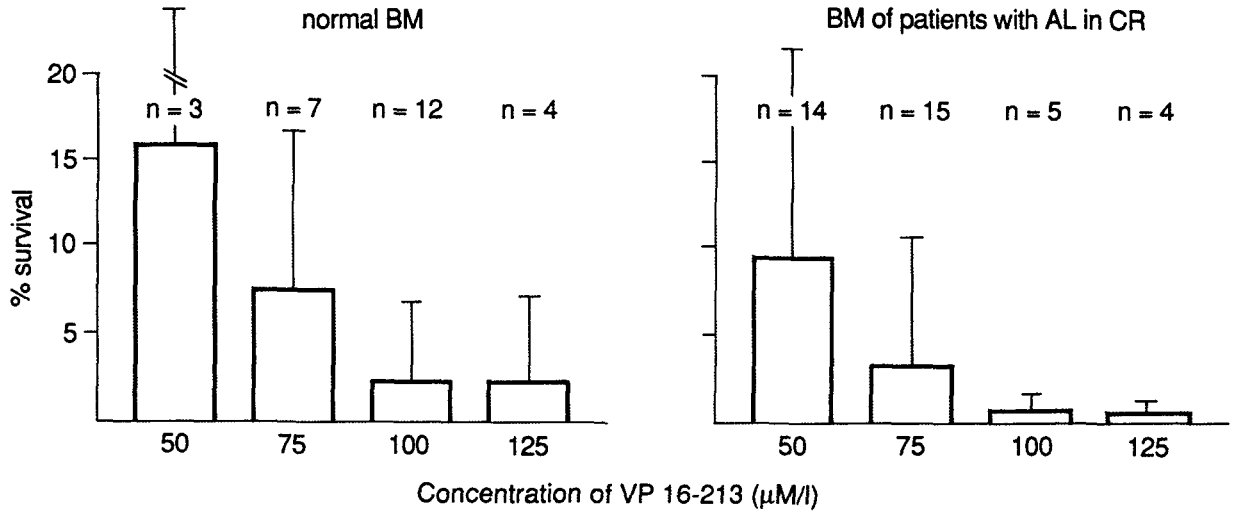

Fig. 1. Recovery at day-0 of GM-CFC after 2-h incubation with VP 16-213

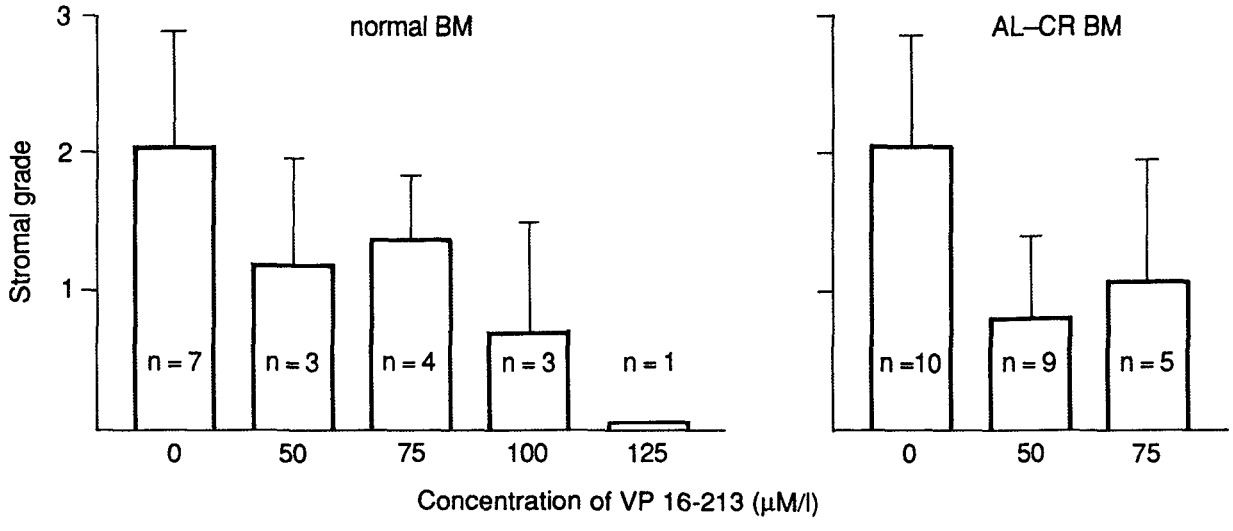

Fig. 2. Degree of adherent layer establishment in 3-week-old LTBMCs of VP 16-213-preincubated bone marrow compared with control cultures. Grade 1, adherent layer only patchy; grade 2, large adherent connected areas; grade 3, surface totally covered

Table 1. Recovery of GM-CFC after one-stage LTBMC of VP 16-213-treated normal bone marrow (% of control)

No. of experiment	Concentration of VP 16 (μ*M*/l)	GM-CFC day 0	GM-CFC after LTBMC
1	100	4.0	30.9
	125	0	1.5
2	50	7.0	14.4
	75	14.0	2.8
3	75	13.5	29.3
	100	0	23.2
4	50	0	6.0
5	100	0	0.1

Table 2. Recovery of GM-CFC after one-stage LTBMC of VP 16-213-treated bone marrow of patients with acute leukemia in complete remission (% of control)

No. of experiment	Concentration of VP 16 (μ*M*/l)	GM-CFC day 0	GM-CFC after LTBMC
1	50	0	10.6
	75	0	7.6
2	50	8.0	0
	75	1.3	0
3	50	17.4	1.0
	75	4.0	0
4	50	12.6	1.0
5	50	0	15.2
6	50	0.3	4.0
7	50	0	0
8	50	1.5	1.0
9	75	0.3	44.9
10	75	6.6	11.3
11	50	0	9.0
	75	0	27.3
12	75	0	0

Table 3. Recovery of GM-CFC after two-stage LTBMC compared with GM-CFC after one-stage LTBMC of VP 16-213-treated bone marrow of patients with acute leukemia in complete remission (% of control)

No. of experiment	Concentration of VP16 (μ*M*/l)	GM-CFC day 0	CM-CFC after 1-stage LTBMC	GM-CFC after 2-stage LTBMC
1	50	0	0	33.8
2	50	1.5	1.0	19.3
3	50	5.1	n.d.	43.1
	75	0.3	44.9	56.2
4	75	6.6	11.3	37.0
5	75	28.1	n.d.	4.8
6	50	0	9.0	18.0
	75	0	27.3	36.0
7	75	0	0	34.0

n.d., Not done

marrow of patients with acute leukemia in complete remission than in normal bone marrow. Whereas the critical dose of VP 16-213 (mean recovery $<5\%$) was 100 μ*M*/l in normal bone marrow, that of complete remission bone marrow was 75 μ*M*/l. However, the differences are not statistically significant.

VP 16-213-treated normal bone marrow showed in all but in one case a better recovery when cultured in one-stage LTBMC for 3 weeks and thereafter assayed for GM-CFC (Table 1). Bone marrow of patients with acute leukemia in complete remission showed an inconsistently different behavior in one stage

LTBMC (Table 2). Six patients had a higher and six a lower recovery compared with GM-CFC on day 0.

It was obvious that the VP 16-213 treatment caused a poorer and delayed establishment of the adherent layer in one-stage LTBMC (Fig. 2). In order to determine whether this might lead to an additional effect on GM-CFC recovery after LTBMC we compared the recovery of one- and two-stage LTBMC. The results are shown in Table 3. With the exception of one experiment, all bone marrow samples showed a distinctly higher recovery in two-stage LTBMC compared with day 0 GM-CFC and also with one-stage LTBMC. It must be pointed out that preirradiated cultures seeded with medium only did not give rise to any hematopoietic growth.

D. Discussion

VP 16-213 is known as a cell-cycle-dependent agent affecting cells in the S and G-2 phases [5, 6]. It shows a strong effect on GM-CFC, a population with a high number of proliferating cells. The possibly higher sensitivity of bone marrow from patients with acute leukemia in complete remission, shown by the lower than normal mean GM-CFC recovery, could be caused by a higher number of proliferating GM-CFC after chemotherapy. The higher recovery of GM-CFC after 3 weeks in one-stage LTBMC of normal bone marrow could indicate less damage to earlier stem cells, containing a lower number of cycling cells. These results agree with those of Ciobanu et al. [1] and Kushner et al. [7], who have also found a higher recovery of post-LTBMC GM-CFC after VP 16-213 incubation.

However, the behavior of bone marrow of patients with acute leukemia in complete remission, the real target of purging procedures, was very inconsistent in one-stage LTBMC after treatment with VP 16-213. This may reflect different answers to the hematopoietic stress of chemotherapy, i.e., a different activation of the early stem cell pool. Otherwise, it was obvious that the establishment of the adherent layer on one-stage LTBMCs was also delayed by drug treatment. Because the maintenance and survival of stem cells in LTBMC depends on an intact adherent layer, representing the hematopoietic microenvironment [8, 9] the possibility cannot be excluded that we measured a resultant of stem cell and stromal effects in our one-stage LTBMC system. To overcome this problem we used the two-stage LTBMC, where the adherent layer is preformed. The result was a much better recovery in this system, so we may assume that our first results with one-stage LTBMC indeed reflected both stem cell and stromal damage.

E. Summary

With a view to the establishment of purging methods it is necessary to investigate complete-remission bone marrow as the real target of purging, rather than bone marrow from healthy donors.

The results of LTBMC are superior to those of GM-CFC where the hematopoietic recovery of bone marrow is concerned. One-stage LTBMC after bone marrow manipulations may reflect mixed hematopoietic/stromal effects. The use of two-stage LTBMC allows the evaluation of the stem cell recovery without the possible influence of a damaged microenvironment.

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