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Protection by Bone Marrow Grafts against Lethal Doses of 5-Fluorouracil*

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Abstract—*C3H (H-2^k) mice were treated with lethal doses of the antimetabolite 5-fluorouracil (5-FU). After 16 hr the mice received an intravenous injection of 25×10^6 syngeneic or allogeneic bone marrow cells. With syngeneic cells, survival after a single dose of 350 mg/kg 5-FU was increased from 27 to 83% and after 450 mg/kg 5-FU from 14 to 84%. Allogeneic CBA (H-2^k) donor cells allowed 81% of the mice to survive after 350 mg/kg 5-FU and 42% after 450 mg/kg 5-FU. Allogeneic marrow from A (H-2^a) donors was ineffective after both doses of 5-FU. The results show that the lethality of 5-FU is largely prevented by a hemopoietic graft. Experimentally, 5-FU belongs to the agents which may be applied in cancer chemotherapy at lethal and thus potentially tumor-eradicative doses in combination with autologous or syngeneic bone marrow. In humans it remains to be seen whether the use of this protocol is precluded by prevailing gut toxicity.*

INTRODUCTION

BONE MARROW cells protect animals and humans from the lethal effects of ionizing radiation. On the other hand, only very few cytotoxic chemicals display a lethality pattern where the toxicity to hemopoietic stem cells predominates in a manner that it can be compensated by hemopoietic reconstitution [1, 2]. The most successful and 'radiomimetic' chemical agent was dimethylmyleran (DMM; dimethylbusulfan; 2,5-dimethanesulfonoxyhexane). Rats [3], mice [4–6], dogs [7] and monkeys [8] survived lethal doses of this drug if they were infused subsequently with bone marrow cells. With regard to other chemicals, busulfan allowed rescue of rats with a hemopoietic graft [9]. Lethal doses of cyclophosphamide could be compensated with bone marrow cells in rats [10] and dogs [11], but not in monkeys [2], where cardiac or gastrointestinal toxicity prevented the hemopoietic rescue of the animals. However, with the vast majority of cytotoxic drugs non-hemopoietic toxicity to other organs, particularly the digestive tract, precludes salvage by bone marrow transplantation. This fact limits the therapeutic value of anticancer agents because it prevents their application at potentially tumor-eradicative dosages, which are likely to be in the lethal or supralethal range. Accordingly, there is a clear need to recognize further cancer chemotherapeutic agents whose lethality can be compensated by bone marrow grafts.

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MATERIALS AND METHODS

Mice

Male C3H (H-2^k) mice (Gl.Bomholtgard, Ry, Denmark), weighing 20–25 g and fed tap water and Nafag pellets *ad libitum*, were used as hosts and syngeneic donors. Allogeneic donor bone marrow was obtained from CBA (H-2^k) and A (H-2^a) mouse donors.

Drugs

5-Fluorouracil (5-FU; courtesy of Dr W. Bollag, F. Hoffman-La-Roche Co., Basel) was dissolved in distilled water and injected by the intraperitoneal route in 0.2 ml/10 g body wt. The LD_{50} of 5-FU was 285 mg/kg. Death after 5-FU occurred between 6 and 15 days.

Bone marrow cell transplantation

Bone marrow cells were prepared from tibiae and femora of donor mice by flushing the bones with TC 199. One donor mouse yielded approximately 50×10^6 cells.

The bone marrow cells were injected intra-

venously to the recipients 16 hr after the 5-FU. They were stored in TC 199 at 4°C and administered intravenously at the dosage of 25 × 10^6 cells in 0.5 ml. The viability of the injected cells was ~95% as determined by the trypan blue exclusion method. Injection of the cells at only 4 hr after the 5-FU led to inferior survival, which is unexpected in the light of the short plasma half-life of 5-FU ($t^1/_2$ = 10–20 min) [12]. The controls received only 0.5 ml TC 199 intravenously.

RESULTS

As shown in Fig. 1, the survival of mice treated with 350 mg/kg 5-FU (a) was increased from 27 to 83% ($P < 0.001$; chi-square test) by the injection of 25 × 10^6 syngeneic bone marrow cells 16 hr after the 5-FU. After 450 mg/kg 5-FU (b) the survival rate was raised with syngeneic bone marrow cells from 14 to 84% ($P < 0.001$). CBA donor cells allowed 81% ($P < 0.001$) of the mice to survive after

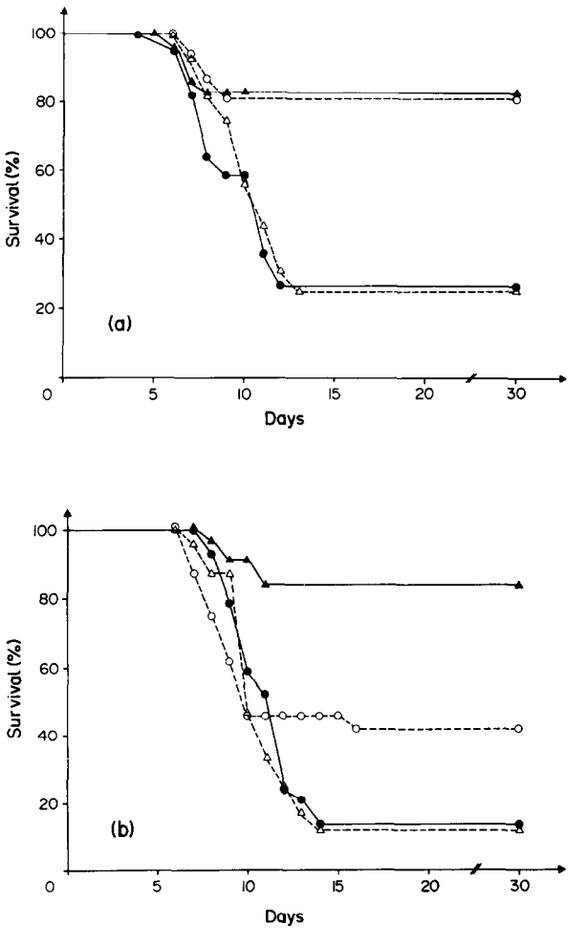

Fig. 1. Survival of C3H mice treated with 350 mg/kg (*a*) *or* 450 mg/kg (*b*) 5-*FU and injected subsequently with* 25 × 10^6 *bone marrow cells of different origin:* (▲——▲) *syngeneic (C3H);* (O- - - - -O) *allogeneic (CBA);* (Δ——Δ) *allogeneic (A);* (●——●) *controls, only TC* 199. *The panels consist of* 16–32 *mice.*

350 mg/kg and 42% ($P < 0.02$) after 450 mg/kg 5-FU. Allogeneic marrow from A donors was ineffective in increasing the survival rate after both doses of 5-FU. No C3H hosts surviving after infusions of either CBA or A marrow showed signs of graft-vs-host disease.

DISCUSSION

The principle of superdose cancer therapy has been applied successfully with lethal radiation in patients suffering from acute leukemia [13, 14]. The possible advantages of lethal chemotherapy followed by bone marrow grafts are the different and perhaps more specific antitumor spectra of cytotoxic drugs and their more general availability. The rationale for single high-dose chemotherapy as compared to conventional multiple application has been documented [15].

Clinically, experience with bone marrow transplantation after superdose chemotherapy is only slowly accumulating. DMM has been used sporadically in the treatment of both hematological malignancies such as chronic granulocytic leukemia [16] and other hematological disorders [17]. Experimentally, human tumor xenografts growing in immunosuppressed mice were eradicated by lethal doses of DMM followed by bone marrow grafts [18]. Other drugs such as melphalan and cyclophosphamide have been used in malignant disease at non-lethal doses with autologous marrow rescue [19–23]. With the exception of DMM, the use of lethal and probably more effective drug doses to eradicate tumors is in general precluded by non-hemopoietic toxicity which cannot be compensated by marrow transplantation.

The observation that marrow after 5-FU is partially effective across weak histocompatibility barriers (CBA → C3H) but not across strong barriers with an H-2 haplotype difference (A → C3H) shows that a single lethal dose of 5-FU is not sufficiently immunosuppressive to permit engraftment of allogeneic donor marrow. This applied also to lethal doses of DMM and is likely to be an advantage for an anticancer agent. Thus, when high or lethal dose therapy with 5-FU is considered, autologous or syngeneic rescue is needed, unless the 5-FU is combined with a potent immunosuppressive measure to permit allogeneic engraftment as does lethal radiation.

The presented results show that 5-FU belongs to the drugs whose toxicity is amenable to bone marrow rescue. This finding coincides with the observation that 5-FU has a generalized killing effect on proliferative hemopoietic cells in the murine bone marrow [24]. With 150 mg/kg of 5-FU a reversible marrow suppression for about 10 days was observed. Our results allow the

conclusion that the more marked and in general irreversible marrow aplasia caused by higher doses of 5-FU can be overcome by marrow grafts.

Preliminary experiments have indicated that single lethal doses of 5-FU followed by syngeneic bone marrow are more effective against human colon cancer xenografts in immunosuppressed mice than multiple tolerated doses of 5-FU. The administration of superdose chemotherapy with 5-FU and bone marrow for sensitive human colorectal carcinomas may be compromised, however, by differences of tissue toxicity of 5-FU between mouse and man and the well-known fact that in the case of humans, the usual dose-limiting toxicity of 5-FU is gastrointestinal, with stomatitis and esophago-pharyngitis occurring in significant numbers of patients at conventional dose levels. It therefore seems unlikely that massive doses of 5-FU could be given to man without prohibitive gut toxicity being produced. More work is needed on survival with autologous marrow in a species where the use of 5-FU is limited by gastrointestinal toxicity.

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