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Most Effective Route of Administration and Utilization of High-Dose Chemotherapy with Bone Marrow Transplantation in Rats[1]

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ABSTRACT

In order to find out which anticancer drugs could utilize to the best advantage a syngeneic bone marrow transplantation in high-dose chemotherapy for cancer, we tested six drugs [nimustine hydrochloride (ACNU), Adriamycin, cyclophosphamide (CY), mitomycin c, vindesin, etoposide] in Sprague-Dawley rats from a standpoint of the beneficial effect of bone marrow transplantation (BMT). Two or three varying doses of each drug were administered i.v. on Day 0, followed by the injection of syngeneic bone marrow (BM) cells (5×10^7, i.v.) on Day 2, and the animals were observed for over 60 days. Adriamycin caused high rates of peripheral neuropathy, and was therefore judged to be inappropriate for high-dose chemotherapy-BMT in this animal model. Among the other five drugs, a beneficial effect of BMT was observed only with CY (300–400 mg/kg) and ACNU (40 mg/kg). In order to enhance the beneficial effect of BMT observed with CY and ACNU, a way of drug administration was designed and carried out. Consequently a higher survival rate was obtained in the following experimental groups: (*a*) (CY 200 mg/kg, Days 0 and 1) + BMT > (CY 400 mg/kg, Day 0) + BMT, (ACNU 20 mg/kg, Days 0 and 1) + BMT > (ACNU 40 mg/kg, Day 0) + BMT. (*b*) (CY 200 mg/kg + ACNU 20 mg/kg, Day 0) + BMT > (CY 400 mg/kg or ACNU 40 mg/kg, Day 0) + BMT. (*c*) (CY 200 mg/kg, Day 0) + (ACNU 20 mg/kg, Day 1) + BMT > (ACNU 20 mg/kg, Day 0) + (CY 200 mg/kg, Day 1) + BMT. Among the six anticancer drugs tested in this study, CY and ACNU were suggested to be more appropriate drugs for high-dose chemotherapy-BMT, but methods for reducing drug toxicity (dose, combination, sequence) were necessary so as to enhance the beneficial effect of the BMT.

INTRODUCTION

The dose-limiting toxicity of most chemotherapeutic agents used to treat malignancies is myelosuppression. ABMT[3] can provide a means to intensify cancer therapy by preventing irreversible marrow failure. The concept of BMT is not new. The first clinical study was reported in 1958 (1). Following this, several studies were completed, but the results were disappointing both in lack of tumor response and protection from myelosuppression. In retrospect, the intensity of chemotherapy was only moderate by today's standards, and the understanding of marrow transplantation was insufficient. There has been a renewed interest in HC with ABMT because of recent developments both in medical technology and the therapy of malignancies (2–9). The HC-ABMT is now actively investigated in many institutions against not only leukemias and lymphomas but also solid tumors. We are also preparing for HC-ABMT against small cell carcinoma of the lung. Before starting this therapy on patients, we carried out animal experiments to find out which anticancer drugs were more appropriate for HC-ABMT from the standpoint of the beneficial effect of ABMT. There has been little informations on this aspect. The present study indicated that CY and ACNU would be suitable candidates for HC-ABMT.

MATERIALS AND METHODS

Animals. Female SD rats were obtained from the Laboratory Division of the Shizuoka Agricultural Cooperative Association in Hamamatsu, Japan. Experimental rats were 12 to 14 weeks old and weighed 210 to 250 g. Four rats were put into one cage and were kept in a clean room. During the experimental period, the cage was exchanged at least three times a week, and antibiotics were administered s.c. at least three times a week for the first month and twice a week for another month.

Anticancer Drugs. ADR, ACNU, CY, MMC, and VDS were dissolved with 0.85% saline solution and administered intravenously through the tail vein. VP-16 was administered i.p. with a total volume of 10 ml in 0.85% saline solution.

Preparation of BM Cells. BM cells were flushed from the femurs of donor animals with a size 22-gauge needle and a 10-ml syringe of Hanks' solution. BM cells were washed twice with Hanks' solution, and 5×10^7 nucleated cells were injected i.v. through the tail vein.

Biochemical Examination. A blood sample was obtained by the cardiac puncture, and the serum was frozen at −80°C until an analysis was made. The biochemical examinations were all commissioned to a certain outside medical laboratory.

Statistics. Lethality was statistically evaluated by Fisher's exact test, and other results were by Student's *t* test. A $P < 0.05$ level of significance was adopted throughout the study.

RESULTS

Effects of SBMT on the Anticancer Drug-induced Toxicity Death in SD Rats. In order to examine the effects of the SBMT on the anticancer drug-induced toxicity death in SD rats, we examined the lethal dose of six anticancer drugs (ACNU, ADR, CY, MMC, VDS, VP-16) used frequently for the treatment of lung cancer (Table 1). When ADR was administered over 8 mg/kg, paresis of the rear legs occurred in almost all the animals approximately 1 month later, which was probably due to the ADR-induced peripheral neuropathy (10). ADR was therefore omitted in the following study, and the other five drugs were tested.

Two or three varying doses of each drug were administered on Day 0, followed by SBMT (5×10^7 cells, i.v.) on Day 2, and the animals were observed for over 60 days. As shown in Fig. 1, a beneficial effect of BMT was only observed in the groups having CY (300, 400 mg/kg) or ACNU (40 mg/kg) treatment. The percentages of animals which died within 3–4 weeks after the drug administration were significantly reduced by means of the BMT in the groups having the CY or ACNU treatment. However, some of the CY-injected animals or almost all of the ACNU-injected ones died after 3–4 weeks without any signs of anemia or hemorrhagic tendency. The beneficial effect of BMT was not observed with MMC, VDS, and VP-16.

Effect of SBMT on the Peripheral Blood Cell Counts in SD Rats Treated with Sublethal Doses of CY or ACNU. Glode *et*

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[3] The abbreviations used are: ABMT, autologous bone marrow transplantation; BMT, bone marrow transplantation; ADR, Adriamycin; MMC, mitomycin c; VDS, vindesin; VP-16, etoposide; BM, bone marrow; CY, cyclophosphamide; WBC, white blood cell; HB, hemoglobin; PLT, platelets; ACNU, nimustine hydrochloride; SBMT, syngeneic bone marrow transplantation; IL-1, interleukin 1; HC, high-dose chemotherapy; MSD, mean survival day; SD rats, Sprague-Dawley rats.

Table 1 *Anticancer drug-induced toxicity death in SD rats*

Drug[a] (mg/kg)	Death/total (%)[b]	MSD[c]	Survival (days) ≦14	~28	~60 day
ACNU					
20	0/7 (0)	60<	0	0	0
30	1/10 (10)	11.0	1	0	0
40	18/20 (90)	19.4	9	6	3
60	8/8 (100)	9.0	8	0	0
ADR					
4	0/5 (0)	60<	0	0	0
8	0/10 (0)	60<	0	0	0
12	5/10 (50)	10.0	4	1	0
CY					
100	0/8 (0)	60<	0	0	0
200	1/11 (9)	9.0	1	0	0
300	13/24 (54)	12.2	10	3	0
400	25/27 (93)	12.1	21	3	1
MMC					
1	0/5 (0)	60<	0	0	0
2	3/15 (20)	36.0	0	0	3
3	9/15 (60)	27.9	0	5	4
4	7/10 (70)	28.4	0	3	4
VDS					
1.5	0/8 (0)	60<	0	0	0
3	7/16 (44)	20.9	5	0	2
4	14/14 (100)	7.8	13	0	1
5	4/4 (100)	4.0	4	0	0
VP-16					
40	0/8 (0)	60<	0	0	0
60	8/9 (89)	19.1	2	5	1
90	5/5 (100)	11.4	5	0	0

[a] Anticancer drug was administered on Day 0, and syngeneic BM cells 5×10^7 were injected i.v. on Day 2.
[b] Death/total (%) was calculated at Day 60 after the administration of anticancer drugs.
[c] Animals which died within 60 days are included.

al. (11) recently reported that ABMT might be useful only in the setting of marrow lethal therapy. Accordingly, we examined the effect of SBMT on the recovery of peripheral blood cell counts in SD rats pretreated with a sublethal dose of CY or ACNU. CY (200 mg/kg) or ACNU (20 mg/kg) was administered i.v. on Day 0, followed by the injection of syngeneic BM cells (2.5×10^7 i.v.) on Day 2, and WBC, HB, and PLT were measured serially. The control values of WBC, HB, and PLT were $120 \pm 24 \times 10^2/mm^3$, 14.9 ± 0.4 g/dl, and $118 \pm 19 \times 10^4/mm^3$, respectively. As shown in Fig. 2, there was a rebounded increase of WBC and PLT in the CY-injected animals, but not with the ACNU-injected ones, and also there was no significant difference in the recovery of the peripheral blood cell count between the BMT and non-BMT groups. It was suggested that the beneficial effect of BMT could be observed clearly only when a lethal dose was administered.

Effects of the Various Ways of Administration of Anticancer Drugs on Toxicity Death. In order to enhance the beneficial effect of BMT observed with CY and ACNU, a way of drug administration was designed and carried out. Firstly, the effects of two half-dose administrations were examined (Table 2 and Fig. 3). In the case of CY-injected animals, there was no significant difference in the lethality and the MSD between the single and two half-dose experimental groups. However, the longest survival time was obtained in the group having two daily consecutive administrations of CY (200 mg/kg) with BMT. In the case of ACNU, the two half-dose administrations of 20 mg/kg showed a tendency of lower toxicity compared with a single administration of 40 mg/kg, and the longest survival time was obtained in the group having two daily consecutive administrations of ACNU (20 mg/kg) with BMT. Again, no beneficial effect of BMT was observed with VDS or VP-16.

Secondly, the effects of combining the sublethal doses of two drugs on toxicity death were examined. A combined sublethal dose of 200 mg/kg of CY and 20 mg/kg of ACNU was administered, and the survival time was compared with that in the group having a lethal dose of 400 mg/kg of CY or 40 mg/kg of ACNU. As shown in Table 3, the lethality was lowest in the group having 20 mg/kg of ACNU plus 200 mg/kg of CY with BMT.

Thirdly, the effects of the administration sequence of two drugs on toxicity death were examined. The following two groups were compared: ACNU 20 mg/kg (Day 0) + CY 200 mg/kg (Day 1), and CY 200 mg/kg (Day 0) + ACNU 20 mg/kg (Day 1). As shown in Fig. 4, the beneficial effect of BMT was observed in the group having CY (0) + ACNU (1), but not in the group having ACNU (0) + CY (1).

Biochemical and Pathological Abnormality Associated with Administration of High-Dose Anticancer Drugs. In order to find out roughly the causes of death in rats having high-dose anticancer drugs, the blood was obtained from the rats which were about to die and biochemical examinations were made. As shown in Table 4, hypoproteinemia, liver dysfunction, elevation of blood urea nitrogen, and electrolyte abnormality, etc., were observed. Several animals were also examined pathologically. The marked interstitial pneumonitis was commonly observed in the groups having CY (300–400 mg/kg) or ACNU (40 mg/kg) treatments (data not shown). Some biochemical abnormalities and interstitial pneumonitis were also observed in randomly selected rats which survived more than 60 days, but lower in frequency and milder in degree of damage (data not shown).

Table 2 *Effects of divided administration of anticancer drugs on the toxicity death in SD rats*

Drug (mg/kg) SBMT[a]	Death/total (%)[b]	MSD
ACNU		
40 × 1 (−)	10/12 (83)	17.6
20 × 2 (−)	5/12 (42)	26.2
40 × 1 (+)	8/12 (67)	34.8[c]
20 × 2 (+)	4/12 (33)	47.5[c]
CY		
400 × 1 (−)	11/12 (92)	10.9
200 × 2 (−)	12/12 (100)	9.4
100 × 4 (−)	12/12 (100)	10.5
400 × 1 (+)	5/12 (42)[c]	26.8[c]
200 × 2 (+)	1/12 (8)[c]	31.0[c]
100 × 4 (+)	4/12 (33)[c]	19.8
300 × 1 (−)	6/9 (67)	11.0
150 × 2 (−)	8/9 (89)	14.6
300 × 1 (+)	3/9 (33)	26.3[c]
150 × 2 (+)	1/9 (11)[c]	26.0[c]
VDS		
4 × 1 (−)	12/12 (100)	8.0
2 × 2 (−)	12/12 (100)	4.0
4 × 1 (+)	10/12 (83)	4.6
2 × 2 (+)	10/12 (83)	4.0
VP-16		
80 × 1 (−)	9/11 (82)	11.8
40 × 2 (−)	10/11 (91)	29.9
80 × 1 (+)	10/11 (91)	10.3
40 × 2 (+)	9/11 (82)	29.4

[a] Syngeneic BM cells 5×10^7 were injected i.v. 2 days after the last drug administration.
[b] Death/total (%) was calculated at Day 60 after the administration of anticancer drugs.
[c] Statistically significant as compared with the SBMT (−) group.

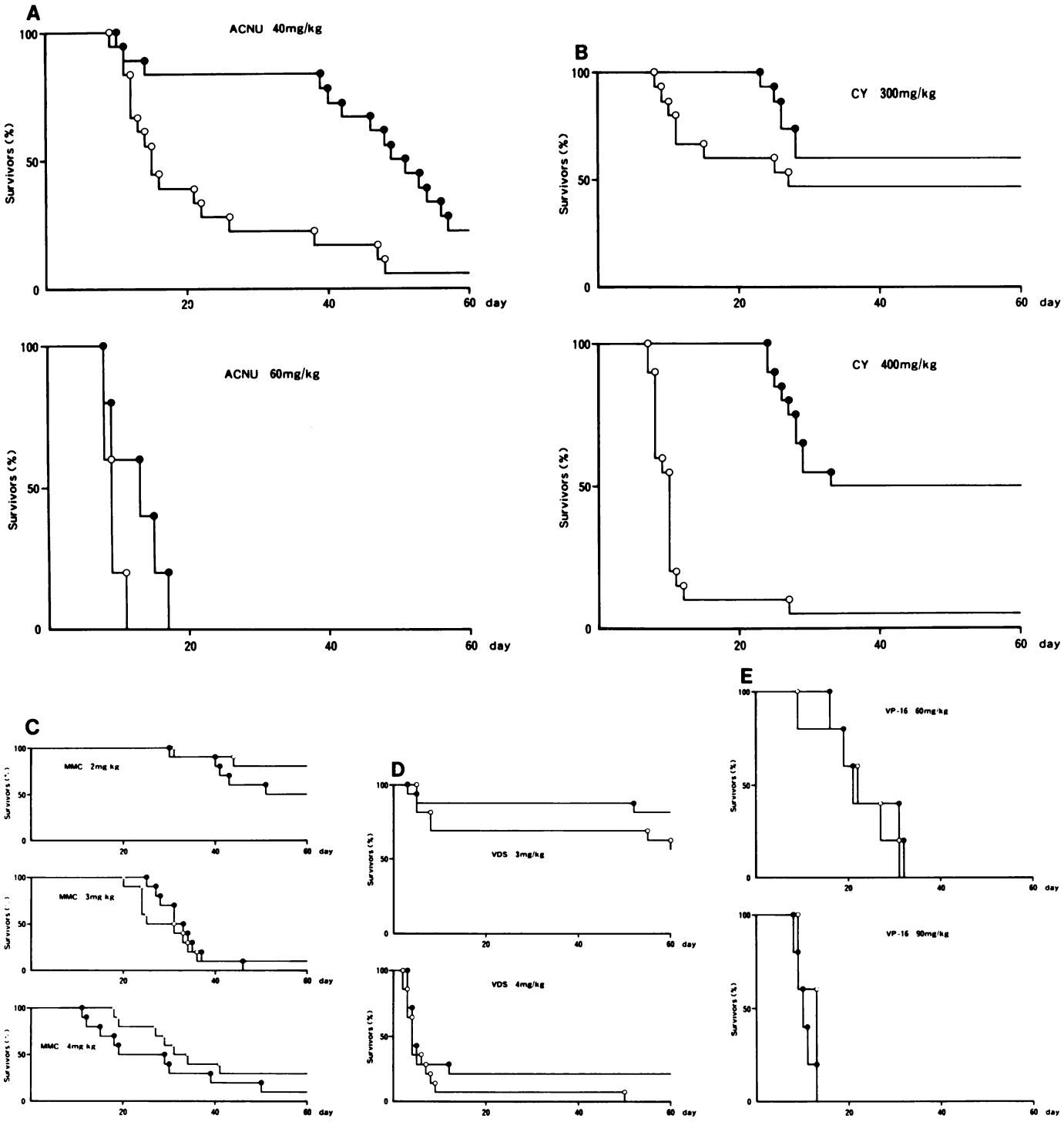

Fig. 1. Survival rates of SD rats treated with high doses of anticancer drugs with or without SBMT. SBMT (+) (●); SBMT (−) (○); *A*, ACNU; *B*, CY; *C*, MMC; *D*, VDS; *E*, VP-16.

DISCUSSION

The therapeutic effect of high-dose chemotherapy with autologous bone marrow transplantation (HC-ABMT) is now actively investigated in many institutions for the treatment of malignancies (2–4, 6–9). However, basic studies on anticancer drugs found to be more appropriate for HC-ABMT from the standpoint of the beneficial effect of ABMT seem to be insufficient. We therefore tested in SD rats six drugs commonly used for the treatment of small cell carcinoma of the lung. Among six drugs tested, the advantage of BMT was observed only with CY and ACNU. The reasons why the beneficial effect of BMT was observed with CY and ACNU, but not with the other drugs have not been intensively investigated yet in our laboratory. The other drugs may be found to have serious toxicities other than myelotoxicity. The characteristic properties of CY and ACNU will be made clear in future studies.

There should exist a certain gap in drug toxicity between humans and animals. For example, ADR-induced neuropathy (10) ascertained by us is not commonly seen in humans. This

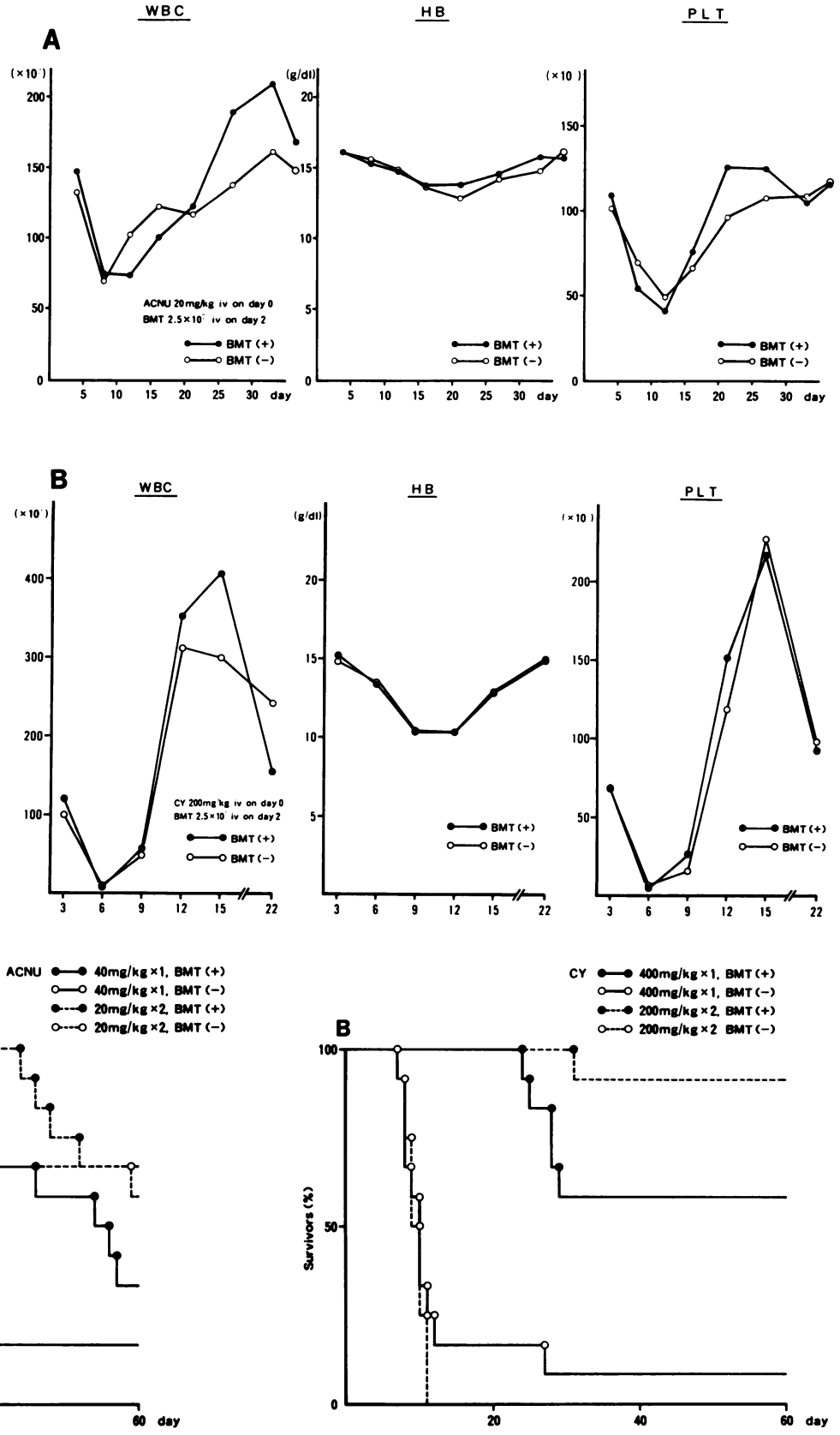

Fig. 2. Changes in peripheral blood cell counts in SD rats treated with ACNU (*A*) or CY (*B*) with or without SBMT. ACNU (*N* = 5), CY (*N* = 8).

Fig. 3. Survival rates of SD rats treated with two half-dose administrations of ACNU (*A*) or CY (*B*) with or without SBMT.

indicates that there is a limitation of using animals for evaluating the drug toxicity, and that there is a great deal of caution while providing some suggestive information. Of course, the common toxicities have been reported; nephorotoxicity by cisplatin (12), pulmonary injury by CY and ACNU (12–16), etc. We suppose that a dose-limiting factor of CY and ACNU in humans may be the interstitial pneumonitis. When toxicities other than myelotoxicity become life-threatening, we should

terminate the dose escalation of the drugs.

In order to reduce drug toxicity, a variety of devices have been reported: cisplatin-induced nephrotoxicity by sodium thiosulfate (17), or by bismuth compounds (18). Concerning myelotoxicity, Neta *et al.* (19) reported that pretreatment with IL-1 could protect mice from radiation-induced death. In preliminary experiments, we tested whether or not IL-1 could reduce CY-induced toxicity death in rats. Pretreatment with IL-1 significantly enhanced CY-induced toxicity death, while some beneficial effect was observed when it was administered after CY treatment. A more detailed study considering dosage and timing of IL-1 is required to reach a conclusion. A granulocyte or granulocyte-macrophage colony-stimulating factor might be useful for enhancing the recovery of bone marrow suppression (20). Some biological response modifiers could be useful for reducing myelotoxicity in certain conditions.

We tested the effects of ways of drug administration on toxicity death. Two half-dose administrations of CY (200 mg/kg) or ACNU (20 mg/kg), or a combination of 200 mg/kg of CY and 20 mg/kg of ACNU resulted in the longer survival time and resulted in the lesser degree in pulmonary toxicity compared with a single high-dose administration of 400 mg/kg of CY or 40 mg/kg of ACNU in the presence of BMT. Interestingly, the lethality of the group having CY (Day 0) + ACNU (Day 1) + BMT was much lower compared with the group having ACNU (Day 0) + CY (Day 1) + BMT. These results indicate that the sequence of drug administration is also important for HC-BMT. The reasons for improved survival rate are unclear. Gregory *et al.* (21) and Millar *et al.* (22) reported that the enhancement of postirradiation recovery of the hemopoietic system was observed in animals pretreated with a variety of cytotoxic agents including CY. They (23) also reported that the lethal effect of high-dose melphalan in mice could be offset by pretreatment with CY, cytosine arabinoside or low-dose melphalan. This was explained by the enhanced regeneration of the hemopoietic system in animals, but the true mechanisms have not been established yet. We are very interested in the reduced lethality observed in the group having CY first and ACNU second in the presence of BMT. A rebounded increase of peripheral WBC and PLT was observed with CY, but not with ACNU (Fig. 2). We are hypothesizing that the rebound phenomenon is somehow related to the observed effect of the administration sequence. Additional studies are now planned to find out the mechanisms of the results shown in Fig. 4. The routes of drug administration were indicated to be important for reducing the drug toxicity and enhancing the advantage of BMT.

In the present study, we demonstrated that some drugs were appropriate and others were not for HC-BMT from the standpoint of the beneficial effect of BMT, and that some techniques were available for reducing drug toxicity. Among drugs tested, only CY and ACNU received the advantage of BMT. In fact, both drugs are now clinically used most frequently for HC-BMT. Many clinicians probably know from their experience that CY and ACNU (or BCNU) are suitable candidates for HC-BMT. For HC-BMT to become a promising and more efficacious therapy in cancer treatment, many more basic studies will be required.

Table 3 *Effects of combining sublethal doses of two drugs on the toxicity death in SD rats*

Anticancer drugs were administered on Day 0, and syngeneic BM cells 5×10^7 were injected i.v. on Day 2.

Drug (mg/kg)	SBMT	Death/total (%)[a]	MSD
ACNU, 40	(−)	9/10 (90)	18.2
CY, 400	(−)	10/10 (100)	9.4
ACNU, 20 + CY, 200	(−)	9/10 (90)	10.7
ACNU, 40	(+)	8/10 (80)	44.0
CY, 400	(+)	5/10 (50)	27.3
ACNU, 20 + CY, 200	(+)	0/10 (0)	60<

[a] Death/total (%) was calculated at Day 60 after the administration of anticancer drugs.

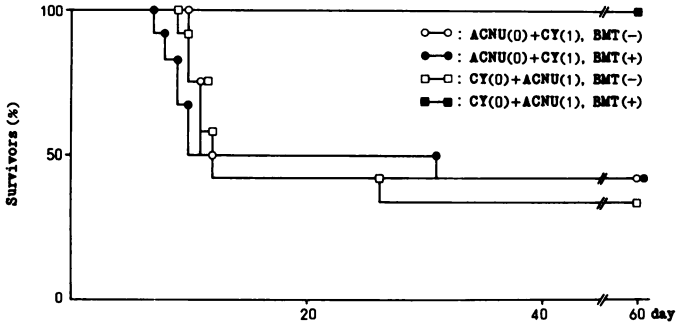

Fig. 4. Survival rates of SD rats treated with CY and then ACNU or ACNU and then CY with or without SBMT. Each group contains 12 rats.

Table 4 *Biochemical data in rats which died within 60 days after administration of high-dose anticancer drugs*

Drug (BMT)	Day[a]	T-P[b] (g/dl)	GOT (IU/liter)	GPT (IU/liter)	Al-P (IU/liter)	BUN (mg/dl)	Cr (mg/dl)	Na (mEq/liter)	Ca (mg/dl)	P (mg/dl)
Normal control ($n = 6$)		7.2 ± 0.4	238 ± 93	57 ± 10	288 ± 72	19 ± 2	0.5 ± 0.1	135 ± 6	9.9 ± 1.2	7.7 ± 1.4
ACNU, 40 mg/kg (+)	40	7.5	384	*105*[c]	158	*34*	*1.2*	151	6.5	*20.4*
ACNU, 40 mg/kg (+)	42	7.0	498	*188*	192	*38*	*1.5*	*158*	6.6	*27.5*
ACNU, 40 mg/kg (+)	48	*5.7*	185	85	150	*35*	0.5	133	8.9	7.5
ACNU, 20 mg/kg ×2 (+)	31	6.2	477	*194*	NT	*42*	0.6	138	NT	12.1
CY, 300 mg/kg (+)	28	*5.6*	*2090*	*712*	145	*40*	0.6	143	*4.9*	*13.3*
CY, 150 mg/kg ×2 (−)	27	*4.3*	84	17	233	62	0.6	151	9.8	7.6
MMC, 4 mg/kg (+)	9	6.7	127	58	124	*137*	*1.0*	122	9.6	6.9
MMC, 4 mg/kg (+)	16	*5.7*	388	*196*	162	*34*	0.3	147	9.5	7.9
MMC, 4 mg/kg (+)	23	6.5	357	*101*	260	*39*	0.3	*159*	9.0	8.1
VDS, 4 mg/kg (+)	9	*4.3*	*2010*	*888*	*604*	*146*	*1.3*	136	8.9	10.6
VDS, 4 mg/kg (+)	11	*3.8*	477	*284*	*904*	*143*	0.6	148	7.6	6.7
VSD, 2 mg/kg ×2 (−)	4	6.6	*7083*	*1584*	295	*166*	*1.7*	133	8.3	*19.3*
VP-16, 80 mg/kg (−)	10	*4.4*	*2820*	*715*	340	*68*	0.5	141	7.4	10.0

[a] Blood samples were obtained from the rats that were about to die.
[b] The abbreviations are: T-P, total protein; GOT, glutamate oxaloacetate transaminase; GPT, glutamate pyruvate transaminase; Al-P, alkaline phosphatase; Cr, creatinine.
[c] Italicized numbers, data which are beyond the range of mean ± 3 SD.

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