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Serum thymidine kinase, a possible marker for monitoring the effect of bone marrow transplant treatment in early recovery phase

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We measured serum thymidine kinase (TK) activity with a radioenzyme assay system employing [I-125]-iododeoxyuridine as the tracer on serial specimens from five bone marrow transplant (BMT) patients before and after transplantation. The serum level of TK activity in the 4 patients with effective BMT treatment ranged from 3.0 to 16.9 U/L (mean, 7.80 U/L) before transplantation and from 27.3 to 236.1 U/L (mean, 82.95 U/L) after the BMT treatment. Mean serum TK activity increased 13.17-fold (range, 1.68 to 29.14-fold). In contrast, the activity in the patient with ineffective BMT treatment was not significantly different during, before, or after BMT treatment. In addition, serum TK activity in BMT patients was well correlated with the change in the number of leukocytes before and after BMT treatment [$r=+0.709$ ($p<0.01$), $y=0.012x+0.87$]. We conclude that the determination of serum TK activity in BMT patients is very useful in monitoring the course of bone marrow transplantation in the early recovery phase.

Key words: serum thymidine kinase, bone marrow transplantation, marker, radioenzyme assay

INTRODUCTION

THYMIDINE KINASE (TK) is an enzyme involved in the introduction of thymidine into deoxyribonucleic acid (DNA).[1] It is found at a very low level in resting cells but is present at a high level in cells preparing to divide.[2] Thus, its presence in a population of cells is a true indicator of the proliferative phase.

In the present study, we investigated the serial change in serum TK activity during the course of bone marrow transplant (BMT) treatment to evaluate its utility in monitoring the effect of the transplantation in the early recovery phase.

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MATERIALS AND METHODS

Patients

We studied five patients (3 males and 2 females) ranging from 17 to 42 years of age (mean, 27.0 years) who had been admitted to our hospital to undergo BMT treatment. All had been clinically diagnosed with hematologic disorders, viz., acute myeloblastic leukemia (AML, 2 patients), chronic myelocytic leukemia (CML, 1), myelofibrosis (MF, 1), and myelodysplastic syndrome (MDS-RA, 1). All patients received the following treatments before BMT treatment: either 60 mg/kg/day of cyclophosphane (CY) for 2 days and 4 mg/kg/day of busulfan (MYL) for 4 days, or 60 mg/kg/day of CY for 2 days and 2.5 Gy/day of total body irradiation (TBI) for 4 days. The patients with MDS-RA received post-treatment consisting of an injection of 250 μg/day of granulocyte colony stimulating factor (GCSF) for 4 days. In all patients, serial blood samples were obtained before transplantation and for 5 weeks

thereafter for measurement of serum TK activity and the number of leukocytes.

Serum TK assay

Serum TK activity was determined with a Prolifigen TK-REA kit (AB Sangtec, Sweden), which is a radioenzyme assay system employing [I-125]-iodo-deoxyuridine as the tracer, kindly supplied to us for clinical trials by Daiichi Radioisotope Labs., Ltd., Tokyo, Japan. The fundamental data for this radio-enzyme assay system, listed below, were obtained by conventional assay procedures in our laboratory. Minimal detectable serum TK activity was 0.63 U/L; multiple dilution of serum from CML patients in the chronic phase yielded curves parallel to those obtained as standards for TK activity; the recovery of TK activity added to serum was 96.3±6.2% (mean and SD); and the standard deviation of interassay and intraassay variation was ±6.0% and ±4.8%, respectively. Clinical trials established the normal range to be 2.3±0.98 U/L (n=49).

RESULTS

As shown in Table 1, BMT treatment was effective in 4 of the 5 BMT patients and ineffective in one. The serum TK activity in the patients with effective BMT treatment ranged from 3.0 to 16.9 U/L (mean, 7.80 U/L) before transplantation and from 27.3 to

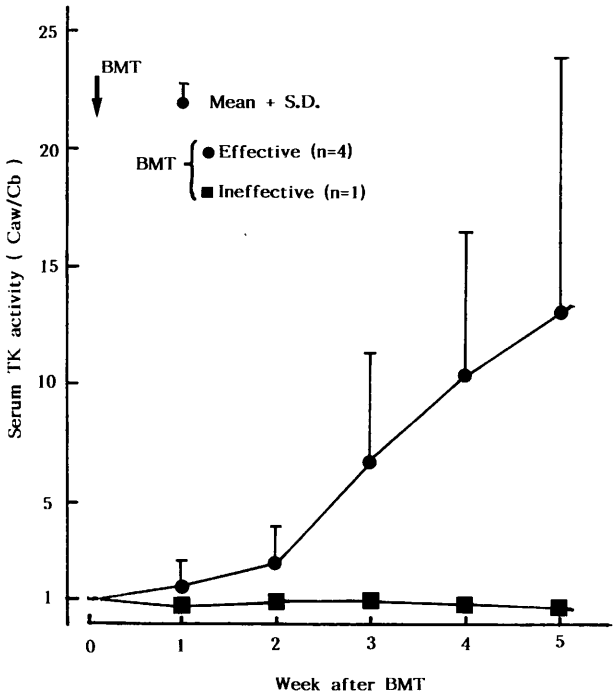

Fig. 1 Time curves for mean Caw/Cb ratio in patients with effective BMT treatment (●) and in the patient with ineffective BMT treatment (■). Caw: mean value of serum TK activity at a given number of weeks after BMT treatment, Cb: serum TK activity before BMT treatment.

Table 1 Summary of clinical characteristics of bone marrow transplant (BMT) patients

Patients	Age (yrs)	Sex	Clinical diagnosis	Pre-treatment	Post-treatment	Peak serum TK level(U/L)		Effect of BMT
						Before BMT(Cb)	After BMT(Ca)	
1 BH	26	M	CML	TBI & CY	–	16.9	28.4 (1.68)#	Effective
2 OT	42	M	AML	CY & MYL	–	3.2	27.3 (8.53)#	Effective
3 CO	26	F	AML	CY & MYL	–	8.1	236.1 (29.14)#	Effective
4 YE	17	M	MF	CY & MYL	–	22.0	21.9 (0.99)#	Ineffective
5 YS	24	F	MDS-RA	CY & MYL	GCSF	3.0	40.0 (13.33)#	Effective

CML : Chronic myelocytic leukemia, TBI : Total body irradiation(2.5 Gy/day for 4 days),
AML : Acute myeloblastic leukemia, CY : Cyclophosphamine(60 mg/Kg B.W./day for 2 days),
MF : Myelofibrosis, MYL : Busulfan(4 mg/Kg B.W./day for 4 days),
MDS-RA : Myelo dysplastic syndrome, GCSF : Granulocyte colony stimulating factor(250 μg/day for 4 days)

( )# : Ca/Cb ratio

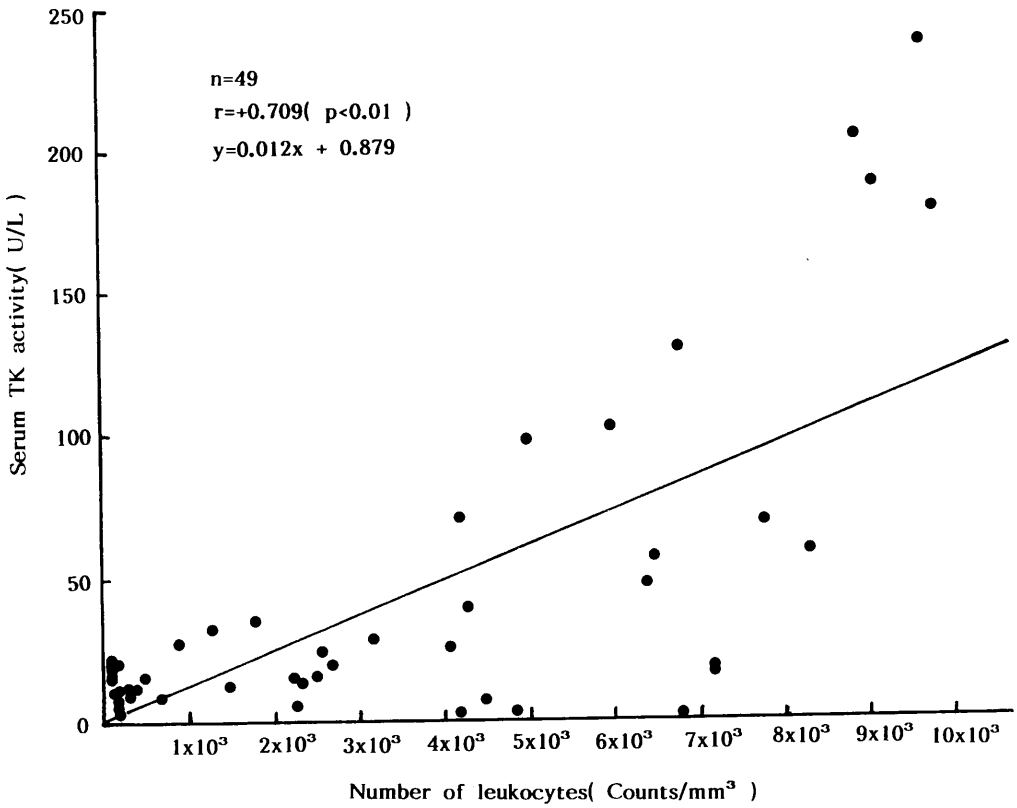

Fig. 2 Correlation between serum TK activity and number of leukocytes in BMT patients before, during, and after BMT treatment.

236.1 U/L (mean, 82.95 U/L) after BMT treatment. Mean serum TK activity increased 13.17-fold after treatment (range, 1.68 to 29.14-fold). In contrast, serum TK activity in the patient with ineffective BMT treatment showed no significant difference before, during or after BMT treatment, being 22.0 U/L before the BMT treatment and 21.9 U/L after the transplantation. The time curve for ratio of mean serum TK activity at each week after BMT (Caw) to serum TK activity before BMT (Cb) (Caw/Cb ratio) increased progressively beginning at 2 weeks in patients with effective BMT; the increase was especially marked after the third week after BMT treatment. In contrast, the time curve for the Caw/Cb ratio in the patient with ineffective BMT showed no significant change during, before, and for 5 weeks after BMT treatment (Fig. 1). In addition, serum TK activity in BMT patients was well correlated with the number of leukocytes before and after the BMT treatment ($r=+0.709$, $p<0.01$, $y=0.012x+0.879$, Fig. 2).

DISCUSSION

Thymidine kinase (TK) is an enzyme involved in the introduction of thymidine into DNA.[1] At least 95% of the TK activity which can be measured in serum exhibits TK type 1 behavior. TK type 1 is one of the cytosolar TKs, and is found mainly in dividing cells and virtually absent in resting cells.[2] The activity of TK 1 in a population of cells is proportional to the proliferative activity of those cells. Therefore, by measuring the serum level of TK activity, the extent of cell division within a population of cells can be calculated. Clinical application of the Prolifigen TK-REA technique was previously reported by several authors in patients with malignant disorders and those with non-malignant disorders.[3–15] Hogberg et al.[12] suggested that the bone marrow cells in patients with B_{12} deficiency have a defect in DNA synthesis, resulting in the accumulation of immature proliferating bone marrow cells locked at the stage of TK production and release. In a study of patients with untreated B_{12} deficiency, serum TK activity was found to be closely correlated with the extent of bone marrow insufficiency, with very high levels being observed in those with the most severe haematological disorders. We therefore studied the utility of measuring serum TK activity in relation to BMT to monitor the effect of transplantation in BMT patients. BMT treatment was effective in 4 of the 5 BMT patients in this study. The serum TK activity

in these patients increased 13.17-fold (mean), with the increase ranging from 1.68 to 29.14-fold, after the BMT treatment. In contrast, no significant change in serum TK activity in the patient with ineffective BMT treatment was observed before, during or after transplantation; TK activity was 22.0 U/L before and 21.9 U/L after BMT treatment. The time curve for the ratio of mean serum TK activity after BMT (Caw) to that before BMT (Cb) (Caw/Cb ratio) for effective BMT patients showed a significant and progressive increase which began at 2 weeks and was especially marked after the third week. It is well known that, during the early recovery phase of BMT treatment, discrete colonies of hematopoietic cells are found in the marrow about 2 weeks after transplantation and that the number of leukocytes usually begins to rise during the third week after transplantation.[16] The data reported in this paper accord with clinical experiential knowledge and, in addition, serum TK activity in the BMT patients is well correlated with the number of leukocytes. In summary, our results indicate that the determination of serum TK activity in BMT patients is very useful in monitoring the effect of the transplantation during the early recovery phase after BMT treatment.

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