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Biochemical Pharmacology 69 (2005) 1585–1594
Biochemical Pharmacology
www.elsevier.com/locate/biochempharm

Relative importance of apoptosis and cell cycle blockage in the synergistic effect of combined R115777 and imatinib treatment in BCR/ABL-positive cell lines

Takuji Miyoshi [a], Tadashi Nagai [a,*], Ken Ohmine [a], Makiko Nakamura [a], Yasuhiko Kano [b], Kazuo Muroi [c], Norio Komatsu [a], Keiya Ozawa [a]

[a] *Division of Hematology, Jichi Medical School, 3311-1 Yakushiji, Minamikawachi-machi, Kawachi-gun, Tochigi 329-0498, Japan*
[b] *Division of Hematology and Medical Oncology, Tochigi Cancer Center, Tochigi, Japan*
[c] *Cell Transplantation and Transfusion, Jichi Medical School, Tochigi, Japan*

Received 13 September 2004; accepted 9 February 2005

Abstract

The combination of imatinib and a farnesyltransferase inhibitor might be effective for reducing the number of BCR/ABL-positive leukemia cells. In this study, we examined the differences in the mechanisms of the growth inhibitory effect of the combination of imatinib and R115777 (Zarnestra™) among BCR/ABL-positive cell lines. Steel and Peckham isobologram analysis indicated that this combination had a strong synergistic inhibitory effect on growth in all imatinib-resistant cell lines and their parental cell lines. Levels of cleaved caspase 3 were increased by the combination treatment in all cell lines. However, both the level of cleaved PARP and the number of annexin-V-positive cells were much less increased in KCL22 and KCL22/SR cells than in K562, KU812, K562/SR and KU812/SR cells. The combination treatment promoted $p27^{KIP1}$ accumulation and induced a significant increase in the percentage of G0/G1 KCL22 and KCL22/SR cells. In other cell lines, the percentage of G0/G1 cells was not increased but rather decreased. The results indicate that induction of apoptosis and blockage of the cell cycle were major mechanisms of the synergistic inhibitory effect of the combination treatment, but the relative importance of these mechanisms differed among cell types. Additional treatment for overriding the G1 checkpoint may be required to eradicate leukemia cells, in which the combination induces cell cycle arrest.

Keywords: R115777; Farnesyltransferase inhibitor; Imatinib; BCR/ABL; Chronic myeloid leukemia; Drug resistance

1. Introduction

The ABL tyrosine kinase inhibitor imatinib mesylate (imatinib, Novartis) has shown a substantial clinical effect in BCR/ABL-positive leukemia patients [1–4]. It has been reported that about 50% of patients with aggressive BCR/ABL-positive leukemia, such as chronic myeloid leukemia in blast crisis (CML-BC) and acute lymphoblastic leukemia (ALL), exhibit a hematological response to treatment with imatinib alone [3,4]. However, most patients with such leukemia relapse soon after showing a response to imatinib; thus, long-term remission is not obtained with imatinib treatment alone. Furthermore, it is possible that many patients with CML-BC will have primary resistance to imatinib because imatinib may already have been administered in the chronic phase in many cases. Previous studies have demonstrated that BCR/ABL gene amplification, point mutations in the ATP-binding pocket of the BCR/ABL gene, increased expression of BCR/ABL protein, up-regulation of P-glycoprotein (P-gp) belonging to the ABC transporter family, increased concentration of serum α1 acid glycoprotein and up-regulation of Nrf2-mediated gene expressions may be involved in the acquisition of resistance to imatinib [5–14]. Several recent studies have indicated that imatinib-resistant cells with a point mutation in the BCR/ABL gene may be present prior to treatment with imatinib in BCR/ABL-positive leukemia patients [5,15–17]. Therefore, to obtain a sufficient clinical effect, it is important to reduce the number of imatinib-resistant leukemia cells by initial treatment targeting aggressive BCR/ABL-positive leukemia. Recently, a new generation of BCR/ABL kinase inhibitors has been developed [18–21] and has been shown to be effective

* Corresponding author. Tel.: +81 285 58 7353; fax: +81 285 44 5258.
E-mail address: t-nagai@jichi.ac.jp (T. Nagai).

0006-2952/$ – see front matter © 2005 Elsevier Inc. All rights reserved.
doi:10.1016/j.bcp.2005.02.021

against imatinib-resistant cells with point mutations in vitro [18]. However, none of these inhibitors are currently available for clinical use. At present, one attractive therapeutic strategy is combination therapy with imatinib and other anti-leukemia reagents. Cytotoxic effects of various combinations on leukemia cells have been investigated [22,23].

Some cellular proteins, including Ras family proteins, require posttranslational modifications to become active. Prenylation, which is involved in these modifications, can be performed by adding a 15-carbon farnesyl isoprenoid group mediated by farnesyltransferase. An alternative prenylation reaction, geranylgeranylation, can be performed by transferring a 20-carbon geranylgeranyl isoprenoid to proteins by geranylgeranyl transferases. Because prenylation is required to transfer Ras proteins to the cellular membrane, farnesyltransferase inhibitors (FTIs) were initially expected to suppress Ras function, leading to tumor growth inhibition [24,25]. An FTI showed significant anti-tumor activity via inhibition of H-Ras function in an activated H-Ras-induced breast cancer model [26]. However, N-Ras and K-Ras can be transferred to the cellular membrane by geranylgeranylation, even if farnesylation is inhibited, suggesting that inhibition of the processing of other target proteins is involved in the anti-tumor effects of FTIs. Such target proteins may include the small GTP-binding protein RhoB and the centromere-associated proteins CENP-E and CENP-F [27,28].

FTIs have been shown to have anti-leukemia effects on BCR/ABL-positive cultured cells and in BCR/ABL-positive murine models [29,30]. Moreover, Hoover et al. reported that an FTI, SCH66336, inhibited proliferation of imatinib-resistant cell lines and colony formation by hematopoietic progenitors from imatinib-resistant CML patients [31]. These findings suggest that FTIs have potential as agents for treatment of imatinib-resistant BCR/ABL-positive leukemia. The results of clinical studies on an FTI, R115777 (Zarnestra^{TM}, Titusville, NJ), indicate that it is moderately effective against CML [32,33]. However, R115777 alone does not seem to be sufficiently effective against aggressive CML [33]. Phase I studies using combination therapy with R115777 and imatinib for treatment of refractory or resistant BCR/ABL-positive leukemia have been conducted [34,35].

In this study, we investigated the mechanisms underlying the inhibitory effect of the combination of R115777 and imatinib on growth of BCR/ABL-positive cells. Our isobologram analysis revealed that this combination has a significant synergistic inhibitory effect on growth of imatinib-resistant cell lines and imatinib-sensitive cell lines. We also found that this effect was due to both induction of apoptosis and blockage of the cell cycle, but the relative importance of these two mechanisms differed among cell lines.

2. Materials and methods

2.1. Cell lines

We previously established an imatinib-resistant clone, KCL22/SR, from the KCL22 human BCR/ABL-positive cell line [36]. To obtain other imatinib-resistant clones, we treated K562 and KU812 cells (BCR/ABL-positive cell lines established from peripheral blood of CML patients in blast crisis) with step-wise increasing concentrations of imatinib (0.1–1.0 μM) and cultured them on a medium containing methylcellulose, followed by selection and cloning of individual colonies. These newly cloned imatinib-resistant cell lines were designated K562/SR and KU812/SR, respectively. All imatinib-sensitive parental cells and imatinib-resistant cells were grown in RPMI1640 medium supplemented with 10% fetal bovine serum and split every 4 days.

2.2. Cytotoxic effects of a combination of R115777 and imatinib

The farnesyltransferase inhibitor R115777 was kindly provided by Johnson & Johnson Pharmaceutical and Development (Philadelphia, PA). Imatinib was purchased from Novartis Pharma (Basel, Switzerland). Cells were incubated with various concentrations of each reagent for 4 days and then cell numbers were counted using a Cell Counting Kit-8 (Wako Pure Chemical Industries Ltd. Osaka, Japan) in accordance with the manufacturer's instructions. The cytotoxic effect of the combination of R115777 and imatinib was evaluated by a Steel and Peckham isobologram as described previously [37,38]. When the points were outside the left margin of the envelope formed by two broken lines, the combination treatment was considered to have a synergistic effect on cell growth inhibition. If the points were plotted within the envelope, the combination treatment was considered to have an additive effect.

2.3. Western blot analysis

Whole cell lysates were prepared from 1×10^7 cells according to a method described previously [39]. Then 10 μg of whole cell lysate was separated electrophoretically using 10% polyacrylamide gel. Immunoblotting and detection by enhanced chemiluminescence were performed as described previously [40]. Mouse anti-glyceraldehyde-3-phosphate dehydrogenase monoclonal antibody and anti-phospho-tyrosine antibody were purchased from Chemicon International (Temecula, CA) and Santa Cruz Biotechnology (Santa Cruz, CA), respectively. Anti-cleaved caspase 3, anti-PARP, anti-p44/42 (ERK1/2) MAP kinase and anti-phospho p44/42 (ERK1/2) MAP kinase rabbit polyclonal antibodies were purchased from Cell Signaling Technology (Beverly, MA). Mouse anti-

$p27^{KIP1}$ and anti-HDJ-2 monoclonal antibodies were purchased from BD Biosciences (San Jose, CA) and Neomarkers (Fremont, CA), respectively.

2.4. Flow cytometry

Apoptotic cells were evaluated by counting annexin-V-positive cells using a MEBCYTO-Apoptosis Kit (MBL, Nagoya, Japan) in accordance with the manufacturer's instructions. Briefly, the cells were collected and rinsed once with phosphate-buffered saline (PBS). The cells were then incubated with annexin-V-FITC and propidium iodide for 15 min and analyzed by flow cytometry using a FACScan Analyzer (Becton Dickinson, San Jose, CA). For cell cycle analysis, the cells were incubated with propidium iodide for 30 min and analyzed by flow cytometry using a FACScan/CellFIT system (Becton Dickinson, San Jose, CA).

3. Results

3.1. Development of imatinib-resistant BCR/ABL-positive cell lines

We used an imatinib-resistant clone, KCL22/SR, and its parental BCR/ABL-positive cell line, KCL22 [36]. In addition, we cloned two other imatinib-resistant clones, K562/SR and KU812/SR, from the BCR/ABL-positive cell lines K562 and KU812, respectively. As shown in Table 1, IC_{50} values of imatinib against the three imatinib-resistant clones were 5–9-fold higher than that against each corresponding parental cell line. No amplification of or point mutation in the BCR/ABL gene was found in these imatinib-resistant clones. Consistent with our previous findings [36], imatinib treatment resulted in a significant decrease in the level of phosphorylation of BCR/ABL protein in all imatinib-resistant clones as well as parental cell lines (data not shown). These results suggest that deregulation of processes downstream of BCR/ABL kinase is involved in the acquisition of resistance to imatinib in these imatinib-resistant clones.

3.2. Combined treatment of BCR/ABL-positive cells with R115777 and imatinib resulted in synergistic inhibition of cell growth

To confirm that the farnesyltransferase inhibitor R115777 inhibits farnesylation in BCR/ABL-positive cells, we examined the level of the chaperone protein HDJ-2, which is a substrate of farnesyltransferase, by Western blot analysis using an anti-HDJ-2 antibody [41]. Treatment of cells with R115777 resulted in significant accumulation of unprocessed HDJ-2 in all cell lines (data not shown), suggesting that farnesylation is effectively inhibited by R115777 in both imatinib-sensitive and imatinib-resistant BCR/ABL-positive cells. To determine whether a combination of R115777 and imatinib effectively inhibits growth of BCR/ABL-positive cells, we examined the time courses of changes in cell count after treatment with IC_{50} concentrations of imatinib, R115777 and a combination of these two reagents. The combined treatment resulted in greater suppression of cell growth than did treatment with either of the reagents alone in all parental and imatinib-resistant cells (data not shown). To determine whether the growth inhibitory effect was synergistic or additive, we next performed Steel and Peckham isobologram analysis, which provides very strict and reliable results [38]. Combined treatment of parental cells (KCL22, K562 and KU812) with R115777 and imatinib resulted in clear synergistic inhibition of cell growth (Fig. 1A). This combination also synergistically inhibited the growth of imatinib-resistant cells, KCL22/SR, K562/SR and KU812/SR (Fig. 1A). These results indicate that the combination of R115777 and imatinib has a synergistic inhibitory effect on growth of BCR/ABL-positive cells, regardless of sensitivity to imatinib.

R115777 was initially expected to be an inhibitor of Ras function. We investigated the levels of phosphorylation of ERK1/2, a Ras-mitogen-activated protein kinase (MAPK), to determine whether the synergistic inhibitory effect was mediated by alteration of Ras signaling. However, the levels of phopho-ERK1/2 were not decreased by R115777 treatment in any of the cell lines (data not shown). These results suggest that inhibition of Ras-MAPK signaling is not involved in the inhibitory effect of R115777 on BCR/ABL-positive cells.

Table 1
IC_{50} values of imatinib against the imatinib-sensitive and the imatinib-resistant cell lines

IC_{50} values(μM)		
KCL22 0.199 ± 0.037	KCL22/SR 1.779 ± 0.934	Ratio ×8.940
K562 0.218 ± 0.091	K562/SR 1.245 ± 0.419	Ratio ×5.711
KU812 0.216 ± 0.076	KU812/SR 1.526 ± 0.308	Ratio ×7.065

3.3. R115777 and imatinib synergistically inhibited the growth of leukemia cells from a patient in blast crisis

We next examined the effect of combined treatment on the growth of primary leukemia cells from a 53-year-old male patient in imatinib-resistant blast crisis. Written informed consent for the examination was obtained from the patient. Leukemia cells from peripheral blood of the patient, with no mutation in the BCR/ABL gene, were used for Steel and Peckham isobologram analysis. The patient showed no response to imatinib after conversion to blast crisis. The IC_{50} of imatinib to these cells was 0.71 μM, which is high compared with those of imatinib-sensitive CML cell lines. Combined treatment of these cells with R115777 and imatinib resulted in a synergistic inhibitory effect on growth (Fig. 1B). These results suggest that this combination treatment is effective against primary imati-

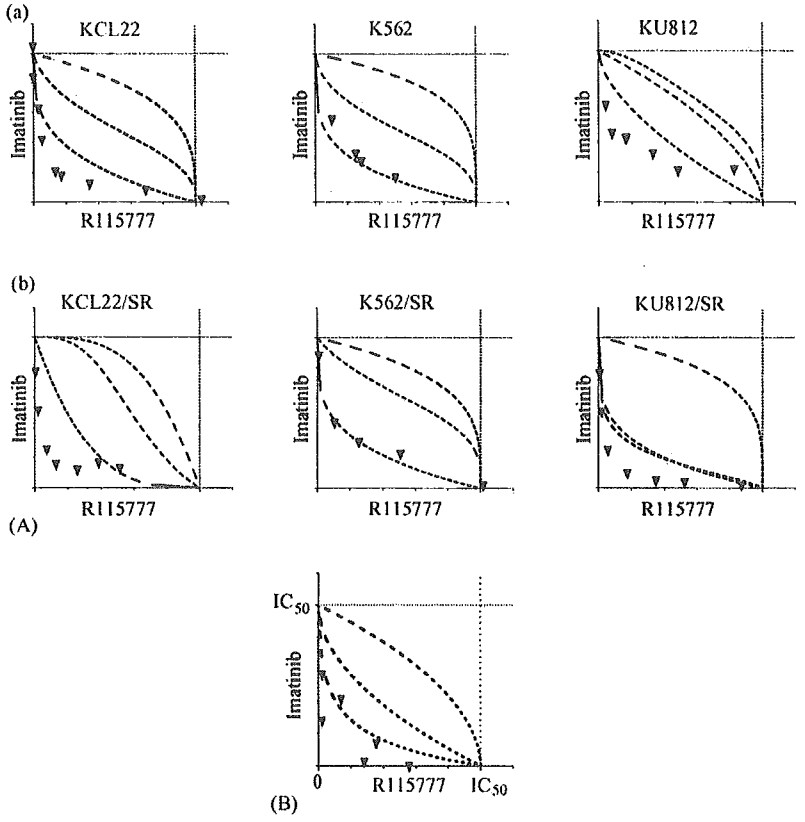

Fig. 1. Effect of combination of R115777 and imatinib on growth inhibition. (A) Steel and Peckham isobologram analyses of the combination of R115777 and imatinib in BCR/ABL-positive cell lines were performed as described in Section 2. Most points are plotted in the area representing synergistic effects in all BCR/ABL-positive parental cell lines (a) and imatinib-resistant cell lines (b). (B) Mononuclear cells from peripheral blood of a patient with imatinib-refractory blast crisis were first seeded at a density of 1×10^5 cells/ml and cultured in RPMI1640 media for 72 h. Steel and Peckham isobologram analysis of the combination of R115777 and imatinib was performed as described in Section 2. Most points are plotted in the area of synergistic effects.

nib-resistant BCR/ABL-positive cells in patients in blast crisis.

3.4. Induction of apoptosis by combination of R115777 and imatinib

To clarify whether the combination of R115777 and imatinib inhibits cell growth due to induction of apoptosis, we examined the levels of cleaved caspase 3, cleaved PARP and the number of annexin-V-positive cells with or without the combination treatment. The combination treatment increased the level of cleaved caspase 3 in all parental and imatinib-resistant cell lines (Fig. 2A). In K562, K562/SR, KU812 and KU812/SR cells, the level of cleaved PARP, which is one of the downstream molecules of caspase 3, was also significantly increased. Consistent with these results, the combination treatment markedly increased the number of annexin-V-positive K562, K562/SR, KU812 and KU812/SR cells, whereas addition of IC_{50} concentrations of imatinib or R115777 alone only slightly increased the number of annexin-V-positive cells (Fig. 2B). In contrast, the level of cleaved PARP was much less increased by the combination treatment in KCL22 and KCL22/SR cells (Fig. 2A). Furthermore, induction of annexin-V-positive cells was much less pronounced in KCL22 and KCL22/SR cells at 72 h (Fig. 2B), 48 h and 96 h (data not shown) after addition of R11577 with imatinib. These results indicate that the combination of R11577 and imatinib induces apoptosis in both imatinib-sensitive and imatinib-resistant cells, but the contribution of apoptosis to the synergistic inhibitory effect on cell growth is relatively low in KCL22 and KCL22/SR cells because of insufficient activation of PARP.

3.5. Effect of the combination of R115777 and imatinib on the cell cycle

Since the combination treatment only slightly increased the number of annexin-V-positive cells in KCL22 and KCL22/SR cells, we hypothesized that the synergistic growth inhibition was mainly caused by induction of cell cycle blockage in these cells. To investigate the function of the G1 checkpoint, we first examined the level of $p27^{KIP1}$. Consistent with our previous findings, $p27^{KIP1}$ expression was up-regulated by treatment with imatinib alone in

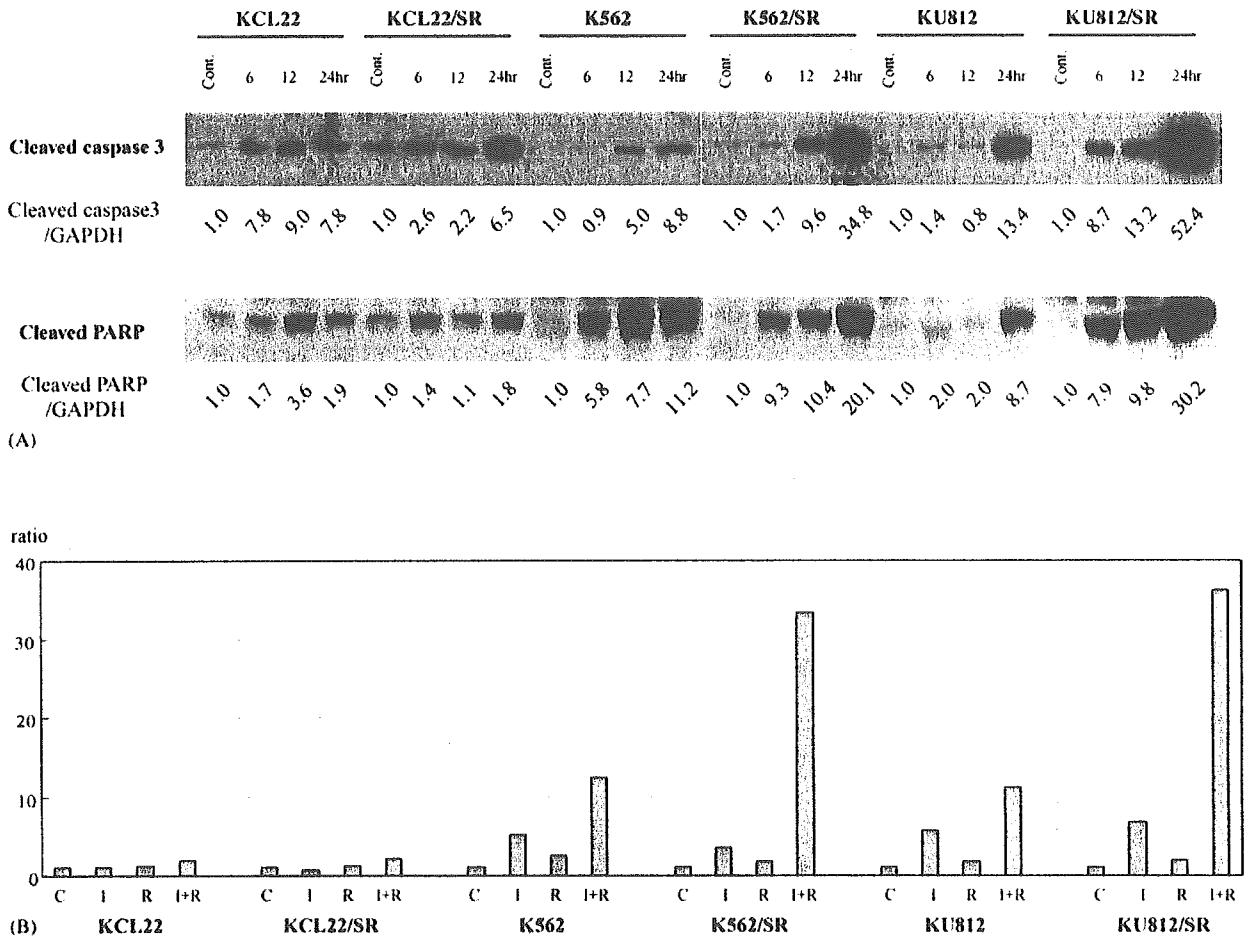

Fig. 2. Induction of apoptosis by a combination of R115777 and imatinib. (A) Cells were cultured in the absence of any reagent for 3 days prior to the treatment and then treated with a combination of IC_{50} concentrations of imatinib and R115777 for 6, 12 and 24 h. Total cell lysates were prepared and subjected to Western blot analysis using anti-cleaved casepase-3 and anti-PARP antibodies. The expression of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was examined as an internal control. The levels of cleaved caspase 3 and cleaved PARP normalized on the basis of GAPDH levels are shown. (B) Cells were cultured in the absence of any reagent for 3 days prior to treatment and then treated with IC_{50} concentrations of imatinib, R115777 or a combination of imatinib and R115777 for 72 h. The number of annexin-V-positive cells was counted by flow cytometry as described in Section 2.

KCL22 and KCL22/SR cells (Fig. 3A). In these cells, the combination treatment with IC_{50} concentrations of R115777 and imatinib also promoted $p27^{KIP1}$ accumulation and significantly increased the percentage of G0/G1 cells (Fig. 3A and B). To determine whether a higher concentration of imatinib could induce cell cycle progression and thus lead cells to apoptosis, we next examined the effect of combined treatment with 5 μM imatinib and IC_{50} concentration of R115777 on $p27^{KIP1}$ expression and G0/G1 accumulation. The results showed that the combination of the reagents at these concentrations increased $p27^{KIP1}$ level and the percentage of G0/G1 cells to the same level and percentage as those in the case of IC_{50} concentrations of R115777 and imatinib (data not shown). These findings suggest that the combination could not abrogate the imatinib-induced activation of G1 checkpoint and that induction of cell cycle arrest rather than induction of apoptosis was thus the main cause of synergistic growth inhibition in KCL22 and KCL22/SR cells. In contrast, the percentage of G0/G1 cells among K562, KU812, K562/SR or KU812/SR cells was not increased but rather decreased by combination treatment (Fig. 3B). Consistent with these results, the levels of cycline D1 were decreased after combination treatment in K562, KU812, K562/SR and KU812/SR cells (data not shown). The $p27^{KIP1}$ level in KU812/SR cells was slightly increased and maintained for 24 h by treatment with imatinib alone, whereas the level was increased at 6 h but declined afterward in K562, K562/SR and KU812 cells (Fig. 3A). Interestingly, combination treatment with R115777 and imatinib had no inhibitory effect on the imatinib-mediated induction of $p27^{KIP1}$ expression in these cells (Fig. 3A). These results suggest that G0/G1 accumulation was not induced in these cells, unlike in KCL22 and KCL22/SR cells, despite G1 checkpoint activation, probably due to the significant induction of apoptosis after combination treatment.

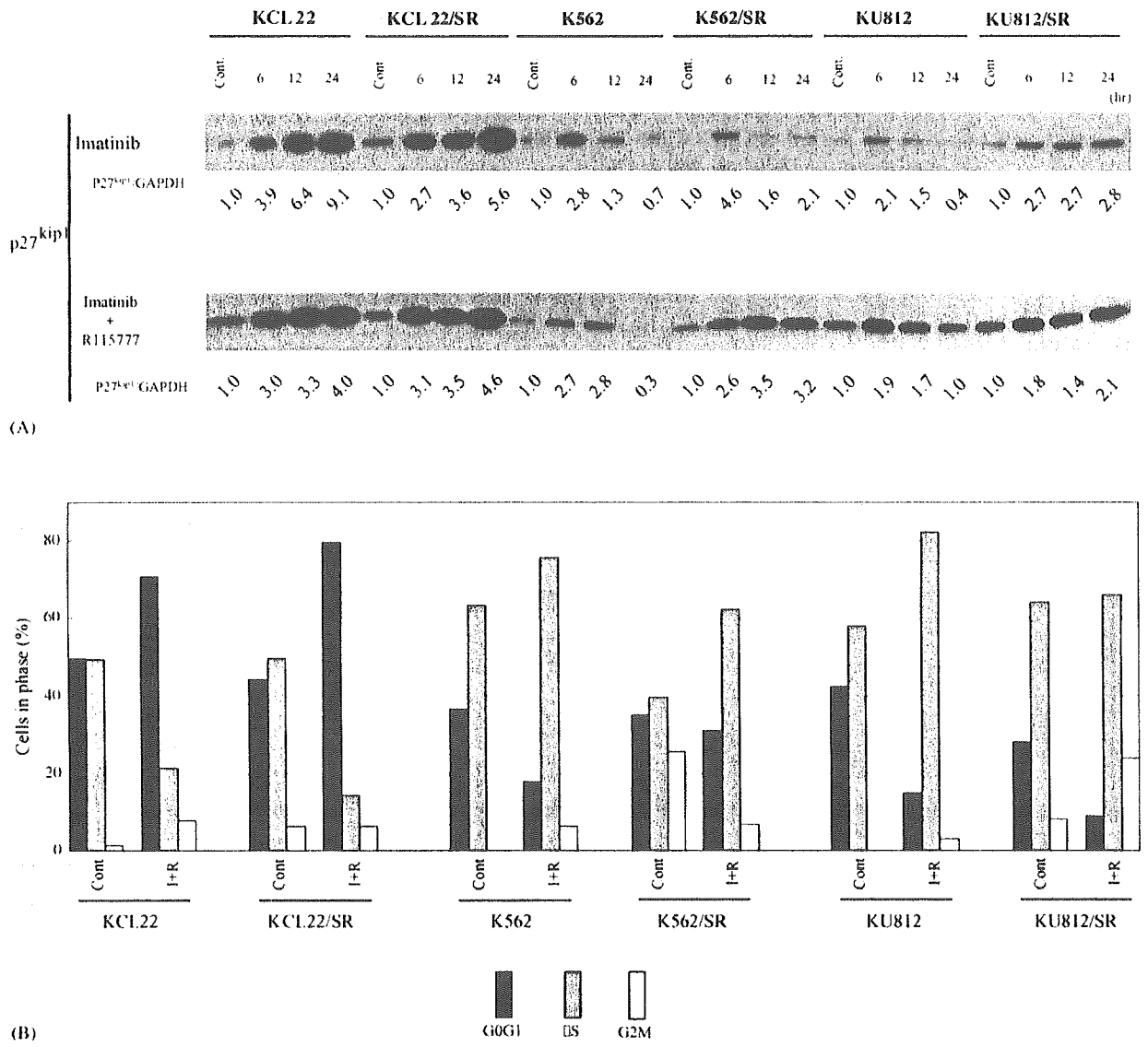

Fig. 3. Effect of combination treatment with R115777 and imatinib on the cell cycle. (A) Changes in $p27^{KIP1}$ protein levels in cells treated with imatinib alone or with a combination of R115777 and imatinib. Cells were cultured in the absence of any reagent for 3 days prior to the treatment and then treated with IC_{50} concentrations of imatinib alone or a combination of IC_{50} concentrations of imatinib and R115777 for 6, 12 and 24 h. Total cell lysates were prepared and subjected to Western blot analysis using anti-$p27^{KIP1}$ antibody. Anti-glyceraldehyde-3-phosphate dehydrogenase (GAPDH) antibody was used as a control for loading (lower panel). (B) Combination treatment of R115777 and imatinib changed the ratios of cell cycle stages. After 24 h of incubation of cells with IC_{50} concentrations of imatinib and R115777, the cells were harvested and incubated with propidium iodide for 30 min and analyzed by flow cytometry with a FACScan/CellFIT system (Becton Dickinson, San Jose, CA).

4. Discussion

Previous studies showed that sustenance of BCR/ABL kinase activity mediated by mechanisms including increased expression of and point mutations in the BCR/ABL gene is a major cause of acquisition of resistance to imatinib [5–14]. In fact, BCR/ABL gene mutations have been found in many clinical imatinib-resistant cases [5–9]. However, there are some cases in which no mutation is found. In the latter cases, deregulation of processes downstream of BCR/ABL kinase may be involved in the resistance to imatinib. Thus, resistance to imatinib can apparently be obtained in both BCR/ABL kinase activity-related and activity-unrelated manners. Imatinib-resistant cell lines examined in the present study exhibited no upregulation of BCR/ABL protein or point mutations in the BCR/ABL gene (data not shown). Moreover, phosphorylation of BCR/ABL was significantly suppressed by imatinib treatment, suggesting that these cells provide a good model of imatinib resistance via a BCR/ABL kinase activity-unrelated mechanism.

FTIs are reagents that may target abnormally activated cellular signaling downstream of BCR/ABL kinase. Previous in vitro studies showed that combinations of FTIs and

imatinib are effective against BCR/ABL-positive cells, but it is unclear whether this effect is additive or synergistic. The present results indicate that combination of R115777 and imatinib synergistically inhibits growth of BCR/ABL-positive cell lines, as indicated by a Steel and Peckham isobologram, which is one of the most reliable methods of analysis for evaluating cell growth inhibition (Fig. 1A). Notably, this synergistic inhibitory effect was also observed in both imatinib-resistant cell lines and leukemia cells from an imatinib-refractory patient (Fig. 1A and B). These results strongly suggest that this combination would have therapeutic value for patients with aggressive BCR/ABL-positive leukemia. It is important to clarify whether the combination treatment is also effective against cells that have resistance-associated mutated BCR/ABL protein, whose kinase activity is not effectively inhibited by imatinib [42]. On the other hand, the contribution of upregulation of P-gp to acquisition of resistance to imatinib is still controversial [43,44]. Fortunately, the effect of the combination treatment may not be influenced by overexpression of P-gp, because the growth of KU812/SR cells (which express P-gp at a level 12.7-fold higher than that in parental KU812 cells) was effectively inhibited by the combination treatment, as was the case with other cell lines.

FTIs were initially developed as inhibitors of posttranslational processing of Ras proteins. However, numerous previous studies suggest that inhibition of the processing of other target proteins such as RhoB, CENP-E and CENP-F is involved in FTI-mediated inhibition of tumor cell proliferation [27,28]. In the present study, R115777 alone had no effect on the levels of phopho-ERK1/2 in any of the BCR/ABL-positive cell lines examined. Taken together with the finding that overexpression of MEK1 (a downstream kinase in the Ras pathway) in KCL22 cells did not restore the cytotoxic effect of the combination treatment (data not shown), this suggests that inhibition of abnormally activated signaling other than Ras-MAPK signaling is involved in synergistic growth inhibition by the combination treatment. We previously found by DNA microarray analyses that RASAP1 and RhoA, which affect or engage in cross talk with cellular signaling, are expressed at higher levels in KCL22/SR cells than in KCL22 cells [36]. It is of interest to clarify whether the effect of the combination treatment is mediated by expression of such molecules.

It has been shown that imatinib induces apoptosis in CML cells [45]. In K562, KU812, K562/SR and KU812/SR cells, R115777 significantly augmented the imatinib-induced increase in the number of annexin-V-positive cells (Fig. 2B). Consistent with these results, the levels of both cleaved caspase 3 and cleaved PARP were increased by the combination treatment. These results suggest that the combination effectively induces apoptosis in these cells. In contrast, the induction of annexin-V-positive cells was extremely low in KCL22 and KCL22/SR cells despite the increase in the level of cleaved caspase 3 by the combination treatment (Fig. 2A and B). One possible explanation for these results is that apoptosis signaling was blocked downstream of caspase 3 in KCL22 and KCL22/SR cells. In fact, the level of cleaved PARP, which is one of the downstream molecules of caspase 3, was much less increased in KCL22 and KCL22/SR cells than in other cell lines (Fig. 2A). Although it is also possible that other unknown mechanisms critically contribute to the blockage of apoptosis, these results suggest that the apoptosis-induction system may break down and that even the combination could not overcome the resistance for the induction of apoptosis in these cells. It is of importance to elucidate the possible unknown mechanisms of apoptosis signaling blockage, and such efforts are now being made in our laboratory.

$p27^{KIP1}$ expression was up-regulated by imatinib alone in all cell lines examined in this study. These results are consistent with our previous findings that imatinib induced cell cycle arrest at the G0/G1 phase, accompanied by up-regulation of $p27^{KIP1}$, in KCL22 cells [46]. Addition of R115777 resulted in no suppression of imatinib-induced up-regulation of $p27^{KIP1}$ expression in all cell lines, suggesting that the combination could not inhibit imatinib-dependent activation of the G1 checkpoint. It is noteworthy that R115777 alone increased the $p27^{KIP1}$ level (in K562, KU812, KCL22 and KCL22/SR cells) or had no effect on the $p27^{KIP1}$ level (in K562/SR and KU812/SR cells) (data not shown). Since FTIs have been shown to induce cell cycle arrest via inhibition of farnesylation of CENP-E protein [47,48], it is possible that CENP-E was a target molecule of R115777 in these cells. Since the apoptosis signal was blocked downstream of caspase 3, the percentage of G0/G1 cells was significantly increased with G1 checkpoint activation after the combination treatment in KCL22 and KCL22/SR cells (Fig. 3A and B). Therefore, it is concluded that cell cycle blockage was mainly involved in the synergistic cell growth inhibition by the combination treatment in KCL22 and KCL22/SR cells. We previously showed that treatment of KCL22 cells with 20 μM imatinib also resulted in G0/G1 accumulation but not in induction of apoptosis [46]. In this study, combined treatment of KCL22 and KCL22/SR cells with R115777 and a higher concentration (5 μM) of imatinib also resulted in G0/G1 accumulation (data not shown). These results suggest that a high concentration of imatinib could not overcome G1 checkpoint activation in these cells.

The other cell lines, K562, KU812, K562/SR and KU812/SR, exhibited different responses. Although the level of $p27^{KIP1}$ was increased by the combined treatment, the percentage of G0/G1 cells was not increased but was rather decreased. The reason for these discrepant phenomena may be the significant induction of apoptosis in these cells. It is likely that apoptosis is induced in the cells before they are led to a G0/G1 state. These results suggest that the induction of apoptosis but not cell cycle blockage plays an important role in the synergistic growth inhibition of K562,

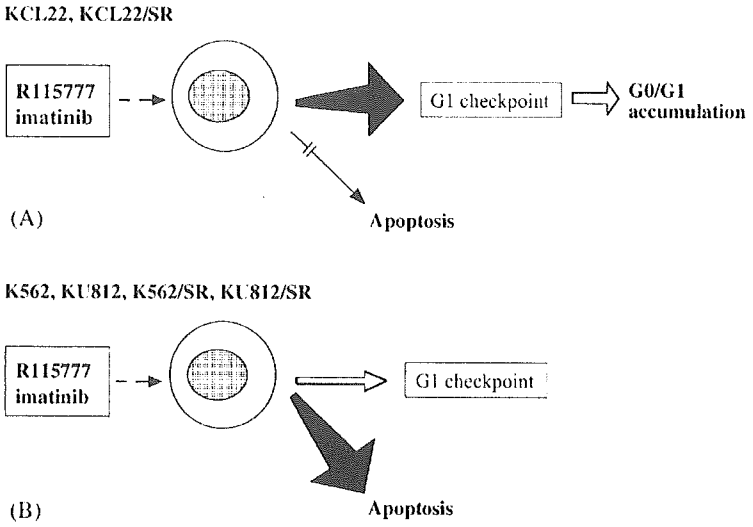

Fig. 4. Hypothetical scheme of the different responses to the combination of R115777 and imatinib in BCR/ABL-positive cells. (A) The combination treatment activates the G1 checkpoint, leading to G0/G1-phase accumulation in KCL22 and KCL22/SR cells, in which apoptosis signaling breaks down. (B) K562, KU812, K562/SR and KU812/SR cells undergo apoptosis with the combination treatment without induction of G0/G1 accumulation.

KU812, K562/SR and KU812/SR cells. A model for the different responses to the combination treatment is presented in Fig. 4. This predicts that the G1 checkpoint remains active but apoptosis signaling breaks down under the condition of combination treatment, leading to G0/G1-phase accumulation in KCL22 and KCL22/SR cells. In contrast, K562, KU812, K562/SR and KU812/SR cells mainly undergo apoptosis by the combination treatment. It is interesting that the imatinib-resistant clone and each corresponding parental cell line showed similar responses to the combination treatment. Therefore, the different pattern of responses might be due to some original cell characteristics, which remain even after acquisition of resistance to imatinib.

The results of this study suggest that the combination treatment of R115777 and imatinib effectively reduce the number of leukemia cells regardless of the sensitivity to imatinib. The finding that the relative importance of the two major mechanisms involved in synergistic inhibition, induction of apoptosis and cell cycle blockage, differed among cell types may have important implications for clinical application of the combination treatment. Since primitive, quiescent BCR/ABL-positive cells may be resistant to imatinib [49], it is likely that KCL22 or KCL22/SR-type leukemia cells, the cell cycles of which are induced to a standstill, may survive after the combination treatment and grow later in the clinical course. Therefore, additional treatment for overriding the G1 checkpoint may be required to eradicate these types of leukemia cells.

Acknowledgments

We are grateful to Drs. Y. Furukawa, T. Kondo, S. Nakano and K. Mitsugi for their helpful discussions. We also thank Ms. E. Yamakawa for her help in preparation of the manuscript. This work was supported by grants-in-aid from the Ministry of Education, Culture, Sports, Science and Technology, Japan.

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© Med Sci Monit, 2005; 11(3): CR91-94
PMID: 15735568

www.MedSciMonit.com
Clinical Research

CR

Received: 2004.09.07
Accepted: 2004.11.08
Published: 2005.03.01

Autologous gamete cryopreservation before hemopoietic stem cell transplantation

Authors' Contribution:
A Study Design
B Data Collection
C Statistical Analysis
D Data Interpretation
E Manuscript Preparation
F Literature Search
G Funds Collection

Takahiro Nagashima[1, 2 ABCDEF], **Kazuo Muroi**[1,2 ABDEF], **Chizuru Kawano-Yamamoto**[1,2 ABD], **Takuji Miyoshi**[2 BD], **Ken Ohmine**[2 BD], **Masaki Toshima**[2 BD], **Akira Miyazato**[2 BD], **Masaaki Takatoku**[2 BD], **Tadashi Nagai**[2 BD], **Masaki Mori**[1,2 BD], **Norio Komatsu**[2 BD], **Keiya Ozawa**[1,2 ADE]

[1] Division of Cell Transplantation and Transfusion, Jichi Medical School, Minamikawachi, Japan
[2] Division of Hematology, Department of Medicine, Jichi Medical School, Minamikawachi, Japan

Source of support: Departmental source

Summary

Background: Infertility after hemopoietic stem cell transplantation (HST) is a serious problem for young patients. Autologous gamete collection before HST may be a promising strategy to overcome infertility.

Material/Methods: From October 1988 to December 2003, six male and nine female patients with hematological malignancies had autologous gametes collected before HST. The data on autologous gamete collection were analyzed.

Results: Sperm could be collected from three patients. However, in two of the three, the numbers and motility of the sperm were severely depleted because they received chemotherapy for one and 11 cycles, respectively. Normal sperm was only collected from one patient with myelodysplastic syndrome who had no history of receiving chemotherapy. One or more oocytes could be collected in five of nine female patients, although the five received multiple cycles of chemotherapy. The successful oocyte collection was associated with an ovulation stimulant.

Conclusions: Autologous oocye collection before HST may be possible, even if patients receive multiple cycles of chemotherapy. In contrast, autologous sperm collection before HST may be difficult after patients receive chemotherapy. Successful pregnancy using autologous gametes after HST remains extremely difficult, especially in female patients; however, it is important to give information on infertility and autologous gamete collection to patients scheduled for HST.

key words: **hemopoietic stem cell transplantation • infertility • autologous gamete collection • unfertilized oocyte**

Abbreviations: **HST** - hemopoietic stem cell transplantation; **IVF** - *in vitro* fertilization; **RIST** - reduced intensity hemopoietic stem cell transplantation

Full-text PDF: http://www.MedSciMonit.com/pub/vol_11/no_3/6362.pdf

Word count: 1397
Tables: 2
Figures: –
References: 20

Author's address: Kazuo Muroi, M.D, Division of Cell Transplantation and Transfusion, Jichi Medical School, Minamikawachi, Tochigi 329-0498, Japan, e-mail: muroi-kz@jichi.ac.jp

Background

HST is an established therapeutic strategy for patients with hematological malignacies and non-malignant disorders. However, patients usually develop severe gonadal dysfunction and subsequent infertility after HST, which are caused by high-dose chemotherapy and/or total body irradiation used as conditioning [1,2]. Infertility is an important problem for patients treated with HST, especially for female patients young enough to become pregnant and hoping to bear a child in the future. In male patients, artificial insemination using frozen autologous semen is easy to perform if a sufficient number of autologous sperm is obtained. If autologous sperms are not available, artificial insemination using donor semen is also possible. In female patients, *in vitro* fertilization (IVF) using frozen autologous fertilized or even unfertilized oocytes has been developed [3–5], although it is difficult to become pregnant for patients with severe gonadal dysfunction caused by high-dose chemotherapy and/or total body irradiation. Another possible approach to create a genetic child is to transfer autologous fertilized oocytes or embryos into the uterus of a related or unrelated healthy woman (surrogate mother) [6]. The Ministry of Health, Labour and Welfare in Japan approves pregnancy of patients with severe ovarian dysfunction using donated oocytes from unrelated women, but not from related women. The office never approves surrogacy.

Previously, several female patients who had undergone HST in our hospital returned to productive lives, but complained of infertility. Since then, we have been clearly informing patients scheduled for HST to expect infertility following conditioning. Those patients were mainly referred to special clinics for collection and cryopreservation of autologous gametes if they wished to undergo that process. We analyzed factors associated with gamete collection in patients scheduled for HST.

Material and Methods

All patients with hematological malignancies and non-malignant disorders planned for HST at Jichi Medical School Hospital from October 1988 to December 2003 were retrospectively analyzed. They were informed of infertility caused by conditioning for HST. After detailed discussion of HST-related infertility, a possible alternative approach to autologous gamete collection and the risks associated with the alternative, patients gave informed consent for autologous gamete collection. Prepubertal patients were not included, but unmarried patients were included. P values below 0.05 were considered significant on student's t test and χ^2 test.

Results

During this period, 48 patients received HST at our institution. Eighteen of 48 patients had cryopreserve autologous gametes collected before HST. Of these, three patients were excluded because of incomplete medical records. As shown in Tables 1 and 2, the patients consisted of six males and nine females and the median age was 24 years (16 to 38 years). Diagnosis included acute myeloblastic leukemia (6 patients), acute lymphoblastic leukemia (3), non-Hodgkin's lymphoma (2), Hodgkin's lymphoma (1), myelodysplastic syndrome (2), and Fanconi anemia (1). Eleven patients had received conventional chemotherapy for their hematological disorders at a frequency of one to 22 cycles before autologous gamete collection. To collect and cryopreserve autologous sperm, all six male patients were referred to one clinic. In females, eight unmarried patients referred to another clinic to collect and cryopreserve autologous unfertilized oocytes using the vitrification method [3,4]. Fertilized oocytes from one married patient were collected and cryopreserved at the Department of Obstetrics and Gynecology, Jichi Medical School Hospital.

Table 1 shows a summary of male patients' profiles and the nature of their collected sperm. In only one patient with myelodysplastic syndrome, normal number and motility sperm were obtained, because he had not received chemotherapy. In the others who had received one or more cycles of chemotherapy, sperm was severely deteriorated or completely absent. Table 2 shows the female patients' profiles and numbers of collected oocytes. In five of nine female patients, one to five oocytes were collected. Of the five, one patient with myelodysplastic syndrome and one with Fanconi anemia had not received chemotherapy before oocyte collection. The other three included two patients with acute myeloblastic leukemia and one with Hodgkin's lymphoma had received multiple cycles of chemotherapy at a frequency of 6 to 15 cycles. All patients who successfully had one or more autologous oocytes received an ovulation stimulant before oocyte collection. There were no significant differences between patients in whom autologous gametes were collected and not collected in terms of age, gender, use of alkylating agents and chemotherapy cycles.

Discussion

We showed that autologous gamete collection before HST may be possible in both male and female patients scheduled for HST. In male patients, the less chemotherapy that had been given, the better the sperm count collected. In female patients, oocyte collection was successfully performed using an ovulation stimulant even if they received multiple cycles of chemotherapy. Although the numbers of pretransplant chemotherapy cycles were not associated with gamete collection statistically, it is recommended that autologous gametes should be collected as soon as possible after achieving complete remission in patients with leukemia or lymphoma.

Pregnancy after HST has been reported in a few patients, in whom gonadal damage has naturally recovered or medical alternatives have been attempted [7–9]. The European Group for Blood and Marrow Transplantation reported that 113 female patients among 37,362 patients receiving HST became pregnant, and 10 of the 113 patients underwent IVF [10]. Three medical approaches are possible to promote pregnancy in female patients after HST: First is IVF using autologous embryos or fertilized oocytes cryopreserved before HST. There are at least two reports of successful pregnancy and live birth using this method [11,12]. This method is clinically available but impractical in very young and single females. It is difficult for patients with malignant hematological disorders to interrupt chemotherapy for autologous oocyte collection. Additionally, it is possible that there will be malignant cell contamination during harvesting of oocytes. Second is IVF using allogeneic donated oocytes af-

Table 1. Sperm collection.

Case	Age (years)	Disease	Chemotherapy numbers	Duration between chemotherapy and collection (months)	Pre- medication	Collected sperm
1	29	AML	11	3	–	Severely disturbed
2	18	NHL	11	3	–	Absent
3	24	MDS	0	0	–	Normal
4	18	ALL	12	0.3	–	Absent
5	16	ALL	1	0.7	–	Severely disturbed
6	38	AML	22	2	–	Absent

AML – acute myeloblastic leukemia; NHL – non-Hodgkin's lymphoma; MDS – myelodysplastic syndrome; ALL – acute lymphoblastic leukemia.

Table 2. Oocyte collection.

Case	Age (years)	Disease	Chemotherapy numbers	Duration between chemotherapy and collection (months)	Pre- medication*	Collected oocyte numbers
7	27	HD	6	12	+	1
8	35	MDS	0	0	+	4
9	23	NHL	8	0.3	–	0
10	22	AML	13	2	+	5
11	29	FA	0	0	+	2
12	17	ALL	6	2	+	0
13	18	AML	0	0	–	0
14	26	AML	12	0**	–	0
15	24	AML	15	2	+	4

HD – Hodgkin's lymphoma; FA – Fanconi anemia;
* ovulation stimulant;
** on therapy with all-trans retinoic acid.

ter HST [13–15]. Although the absence of malignant cell contamination risk during harvesting of the oocytes is a clinical benefit, it is difficult to obtain donated oocytes. In Japan, oocyte donation only from unrelated, but not related, women to patients with gonadal dysfunction is approved. Gestational surrogacy is a treatment option available to women with certain medical problems including severe gonadal dysfunction [6]. Gestational surrogacy arrangements are carried out in a few European countries and in the USA. However, ethical or legal problems remain unresolved and gestational surrogacy is not approved in Japan. Third is cryopreservation of autologous unfertilized oocytes before HST. This method could be applied to female patients with or without husbands, and could be a good option from the perspective of medical ethics, although cryopreservation of unfertilized oocytes is thought to be difficult. Recently, a new technique, called vitrification, has been developed, which does not cause intracellular ice formation in oocytes during cryopreservation [3,4]. There are three reports of successful pregnancy and live birth after implantation of autologous or donated unfertilized oocytes using the vitrification method [4,16,17]. Very recently, it has been reported that follicle development and estrogen production of a breast cancer patient return to normal following cryopreserved autologous ovarian tissue transplantation [18]. In patients with hematological malignancies, ovarian tissue cryopreservation may be difficult, since there will be malignant cell contamination.

We do not know whether reduced intensity hemopoietic stem cell transplantation (RIST) preserves gonadal function at a normal level. Recently, Kyriacou et al. reported that a reduced intensity regimen of fludarabine, melphalan and CAMPATH-1H induced damage to germ cells and Leydig cells [19]. If RIST using other regimens does not impair gonadal function and shows the same clinical outcomes as conventional intensity hemopoietic stem cell transplantation for young patients with standard-risk leukemias or lympho-

mas, such RIST may be a better strategy for patients hoping to have their own childen in the future.

Although approaches to becoming pregnant after HST may differ country by country based on medical ethics [20], we should provide the correct information on autologous gamete collection to patients scheduled for HST. Such information, along with autologous gamete cryopreservation, increases the fighting sprit of young patients scheduled for HST, even though successful pregnancy using autologous gametes after HST remains extremely difficult.

Conclusions

1. Autologous gamete collection is possible in both male and female patients before HST.

2. Information on autologous gamete collection should be given to patients scheduled for HST.

Acknowledgements

We would like to thank nurses of the hemopoietic stem cell transplantation team at Jichi Medical School Hospital for their excellent contribution regarding information on infertility.

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8．間葉系幹細胞の造血幹細胞移植への応用

室井　一男*
Muroi　Kazuo

自治医科大学輸血・細胞移植部　助教授

Summary　骨髄細胞中の間葉系幹細胞（MSC）は，容易に分離し体外で増幅させることができる。MSCは，非上皮細胞への多分化能，造血幹細胞の支持能，強い免疫修飾作用を有するユニークな細胞である。これらのMSCの機能に基づき，造血幹細胞移植に関連したMSCを用いた臨床試験が行われており，有望な結果が得られつつある。MSCを用いた臨床試験では，MSCの投与に関連した重篤な副作用がみられていないことも大きな特徴である。

はじめに

間葉系幹細胞（mesenchymal stem cell：MSC）は，骨髄細胞中に存在するplastic dishに付着する線維芽様の細胞で，*in vitro*で容易に増殖させることが出来る[1]。MSCは造血幹細胞に発現するCD45，CD34，CD117，CD133が陰性で，CD73（SH3），CD105（SH2），SSEA4等が陽性であり，骨，軟骨，脂肪細胞，筋細胞，血管内皮や神経へ分化する能力を有する細胞である[1]。血液疾患に対するMSCを用いた臨床試験では，①MSCの分化能を利用して，障害された細胞の修復に用いる，②MSCの造血幹細胞支持能を利用して，自家または同種造血幹細胞移植時の生着と造血促進に用いる，③MSCの免疫修飾作用を利用して，同種造血幹細胞移植時の移植片対宿主病（graft-versus-host disease：GVHD）予防や治療に用いる3つが行われている。本稿では，これらの臨床試験について順次解説する。

1．先天性代謝異常症と骨形成不全症に対するMSCの投与

先天性代謝異常症の中でもlysosome病は，必要な酵素の先天的欠損によってlysosome内に不要な，あるいは有害な物質が蓄積する疾患である。造血幹細胞移植はlysosome病に対して有効であるが，造血幹細胞移植後に産生された正常なリンパ球や単球が損傷された組織に到達し，欠損している酵素を細胞外に分泌し，分泌された酵素

《略語一覧》
MSC（mesenchymal stem cell；間葉系幹細胞）
GVHD（graft-versus-host disease；移植片対宿主病）

を障害された(酵素を持たない)細胞が取り込むことで蓄積された異常な物質が低下するためと考えられている。MSCをlysosome病の治療に用いる場合には，①移植したMSCが損傷した組織で正常な細胞に分化する，②移植したMSCから分化した細胞が欠損している酵素を分泌する2つの機序が考えられる。Huler症候群は，α-L-iduronidaseの欠損のため，heparan sulfateとdermatan sulfateがlysosomeに蓄積し，肝脾腫，心疾患，骨格異常，水頭症や精神遅滞を来す疾患である。異染性白質ジストロフィー（metachromatic leukodystrophy：MLD）は，arylsulfatase A欠損のためsulfatidesの蓄積し，中枢神経と末梢神経の脱髄のため歩行困難，四肢麻痺や精神遅滞を引き起こす。Kocらは[1]，以前骨髄移植を受けた5人のHuler症候群と6人のMLDに，同一ドナーの骨髄細胞から得られたMSCを，体重当たり2.0×10^6から10.0×10^6細胞を投与し臨床効果を検討した。副作用は認められなかった。MSC投与後の骨髄細胞のキメリズム検査では，day 60の時点で，2例に0.4%と2.0%のドナー由来のMSCが確認された。骨密度の改善は軽度であったが，4人のMLDで神経伝達速度の改善を認めた（図1）。残念ながら，精神や身体の発達には変化を認めなかった。骨形成不全症は，骨形成の主要な蛋白であるⅠ型collagenが形成されないため生じる疾患で，多発性の骨折を来しやすく四肢の変形や短身長を生じる。Horwitzらは[3]，骨髄移植を受けた6人の小児に，同一のドナーの骨髄細胞からMSCを分離し増殖させた後，neomycin耐性遺伝子を組み込んだretrovirusをMSCにtransfectionした。骨髄移植後18～34カ月を経過した6人の子どもに，遺伝子マーキングしたMSCを体重当たり1.0×10^6から5.0×10^6細胞を投与した。重篤な副作用は認められず，5人では骨，皮膚や骨髄stormaに移植したMSCの存在が確認された。臨床効果は明らかで，MSCの生着が確認された5人では，MSCの移植6カ月後の成長速度は，同年齢の子どもの60～94%に回復した(図2)。MSCは，lysosome病や骨形成不全症に有効である可能性が示された。

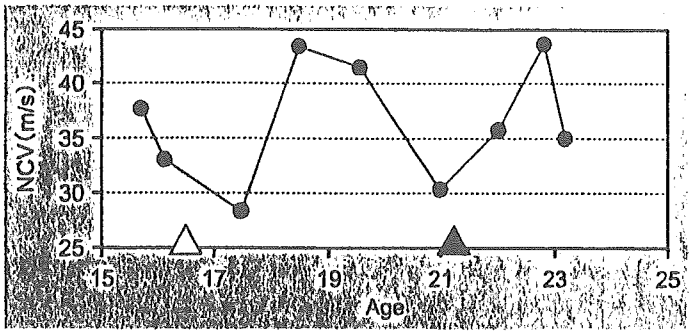

図1　MSC投与後の神経伝達速度の変化
MLDの患者に骨髄移植（△）を行ったところ，peroneal nerveの神経伝達速度は改善したが，移植約4年後低下した。同一ドナーからのMSCを投与（▲）したところ，神経伝達速度は再び改善した。
（文献2より引用）

2．造血幹細胞移植時の生着促進を目的としたMSCの投与

MSCは骨髄微小環境を構成し，造血幹細胞の支持能を有するので，造血幹細胞移植時にMSCを同時投与すると，移植した造血幹細胞の生着と造血回復を促進する効果が期待される。Kocらは[1]，32人の乳癌の患者の骨髄細胞からMSCを分離し増殖した。28人の患者で，自家末梢血幹細胞移植後の1または24時間後に体重当たり1×10^6細胞以上の自家MSCを投与した。MSC投与に関連した副作用は認められなかった。移植後の造血の回復は速やかで，好中球が500/μLを超え

《略語一覧》
MLD（metachromatic leukodystrophy；異染性白質ジストロフィー）

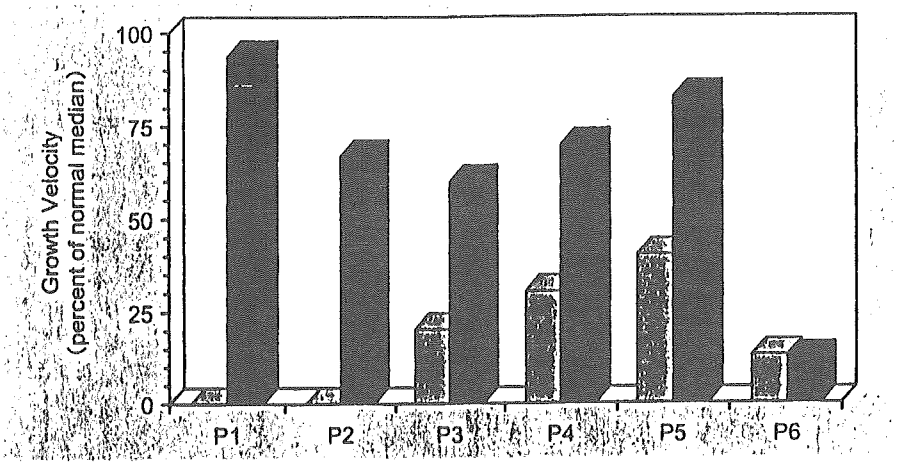

図2　MSC投与後の身長の伸びの速度

骨形成不全症に対して骨髄移植を行い，その後，同一ドナーからMSCを投与した。MSCの投与前（灰色）とMSCの投与6カ月後（黒）に，身長の伸びを比較したところ，投与後にMSCの存在が確認された5人（P1～P5）では，身長の伸びる速度は増加した。

（文献3より引用）

る日は移植後8日，血小板が2万/μLを超える日は移植後8.5日であった。

近年，臍帯血移植は盛んに行われ，本邦における非血縁者造血幹細胞移植の約半分を占めるようになった。臍帯移植で問題となるのは，移植細胞数の少なさが原因と考えられる生着遅延や拒絶である。複数の臍帯血を同時に移植する試みが行われているが，複数の臍帯血を同時移植することによって，移植細胞数を増加しても，移植後の血球の著明な回復促進は生じないこと，原因として生着後どちらか一方の臍帯血由来の造血が優位となることが報告されている[5]。最近，Kimらは[6] 2種類のヒト組織適合抗原(human leukocyte antigen：HLA）が3～6座不一致の臍帯血を，CD34陽性細胞数を同数含む単核細胞に調整後，第3者から得られたMSCと一緒にnon-obese diabetic/severe combined immunodeficient（NOD/SCID）マウスに移植し，short tandem repeatマーカによる移植後のキメリズムの割合を検討した。MSCを同時に移植すると，2種類の造血がほぼ等しく起こること（表1），ヒト由来のCD71（transferrin receptor）陽性の増殖細胞が約2倍に増加すること（図3）を報告した。このMSCの作用は，2つの臍帯血中に含まれるリンパ球のgraft-versus-graft reactionを抑制することによって，各々の造血幹細胞の生着が促されたと考えられた。

3．GVHD予防または治療を目的としたMSCの投与

MSCは，mitogen刺激やmixed lymphocyte reactionによるT細胞の活性化を強く抑制することが知られている[7]。最近，MSCは成熟した樹状細胞（DC1）に作用し腫瘍壊死因子（tumor necrosis factor：TNF）αの産生を抑制すること，成熟

《略語一覧》
HLA（human leukocyte antigen；ヒト組織適合抗原）
NOD/SCID（non-obese diabetic/severe combined immunodeficient）
DC（dendritic cells；樹状細胞）

表1　2種類の臍帯血同時移植後のキメリズム

臍帯血移植	移植したマウス(数)	ドナーA(%)	ドナーB(%)	A：B
A＋B	26	73.5 ± 3.1	26.5 ± 3.1	2.8：1
(A＋B)＋MSC	19	64.5 ± 2.7	35.5 ± 2.7	1.8：1

NOD/SCIDマウスに，同数のCD34陽性細胞を含むAとBの2種類の臍帯血を同時移植し，移植後のヒト細胞のキメリズムを検査した。AとBを同時移植すると，どちらか一方の臍帯血(表の場合にはA)の造血が優位となるが，MSCを同時投与すると，AとBの造血がほぼ等しくなる。　(文献6より引用)

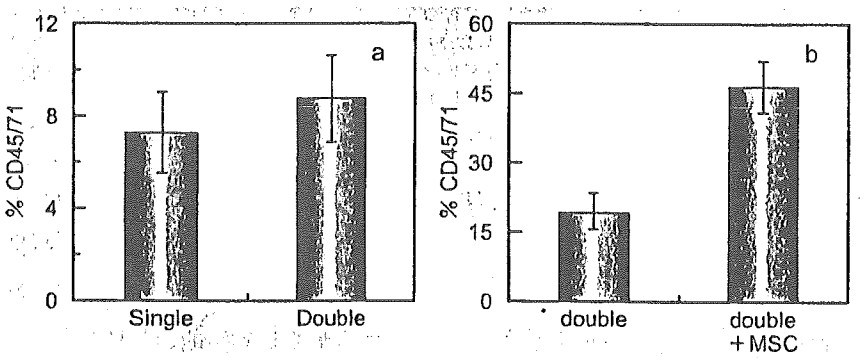

図3　臍帯血移植の生着に及ぼすMSCの効果

同数のCD34陽性細胞数を含む臍帯血単核細胞2種類を，NOD/SCIDに同時移植(double)した。臍帯血のみの移植(double)では移植CD34陽性細胞数を2倍にしても，移植後の生着細胞の増加は認められなかった(a)が，MSCを一緒に移植すると(double＋MSC)，生着する細胞数は約2倍に増加した(b)。　(文献6より引用)

した樹状細胞(DC2)に作用しinterleukin(IL)-10の産生を増加させることによって，Th1細胞からのIFNγ産生を抑制しTh2細胞からのIL-4産生を誘導することが示された(図4)[8]。また，MSCはregulatory T細胞を増加させ，natural killer(NK)細胞からのIFNγ産生を抑制した(図4)。また，MSC自身の抗原性は低いことが報告されている。このようなMSCによる免疫修飾作用は，GVHDの予防や治療に有効である可能性がある。

米国のベンチャー企業のOsirisは，第3者の骨髄細胞から得れたMSCを，HLA一致ドナーからの骨髄移植時に同時投与し，GVHD発症率，移植関連死亡(transplant related mortality：TRM)，生存率を比較する臨床試験を行った(図5)。MSCを投与した群では，慢性GVHDの頻度の低下，TRMの低下，生存率と無病生存率の増加が認められた。MSC投与による重篤な副作用は認められず，MSCは同種造血幹細胞移植後のGVHD予防に有用と考えられた。本邦においても，HLA一致ドナーからの骨髄移植時に，GVHD予防を目的として非血縁者MSCを同時投与する第1相臨床試験が計画されている。最近，

《略語一覧》
TNF (tumor necrosis factor；腫瘍壊死因子)
IL (interleukin)
NK (natural killer)
TRM (transplant related mortality；移植関連死亡)

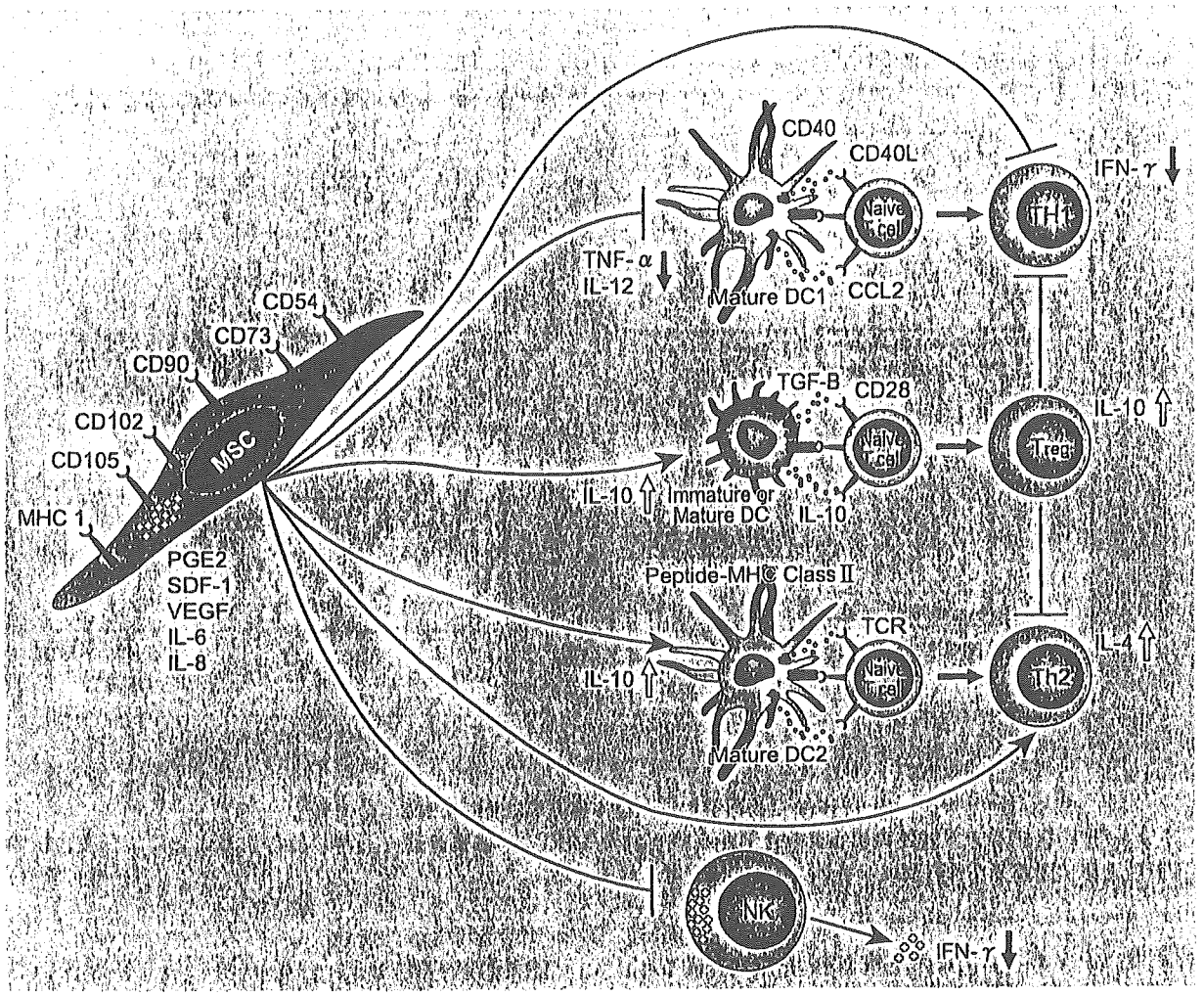

図4　MSCの免疫修飾作用

MSCは，樹状細胞のTNFαの分泌を抑制し，IL-10の分泌を促進することによって，樹状細胞の成熟と機能に影響を及ぼし，その結果として抗炎症作用と免疫寛容をもたらすと考えられる。

死亡（transplant related mortality：TRM）

（文献8より引用）

興味ある症例報告がなされた（図6）[9]。症例は9歳の急性リンパ性白血病（acute lymphocytic leukemia：ALL）男児で，第3寛解期にHLA一致の非血縁女性ドナーから，同種末梢血幹細胞移植を受けた。移植後，急性GVHD（皮疹，下痢と腹痛，黄疸）が出現した。各種治療（psoralen with ultraviolet-A light〔PUVA〕，methylprednisolone，inflixmab，daclizmab，mycophenolate mofetil，methotrexate）を試みたにも係わらずⅣ度の急性GVHDに進展した。そこで，児の母親の骨髄からMSC分離し増殖させ，体重当たり2×10^6個のMSCを投与した。投与後，速やかな血清ビリルビンの低下と下痢の減少が認められた。その後，骨髄で微小残存白血病を認めたため，移植片

《略語一覧》

ALL（acute lymphocytic leukemia；急性リンパ性白血病）

PUVA（psoralen with ultraviolet-A light）

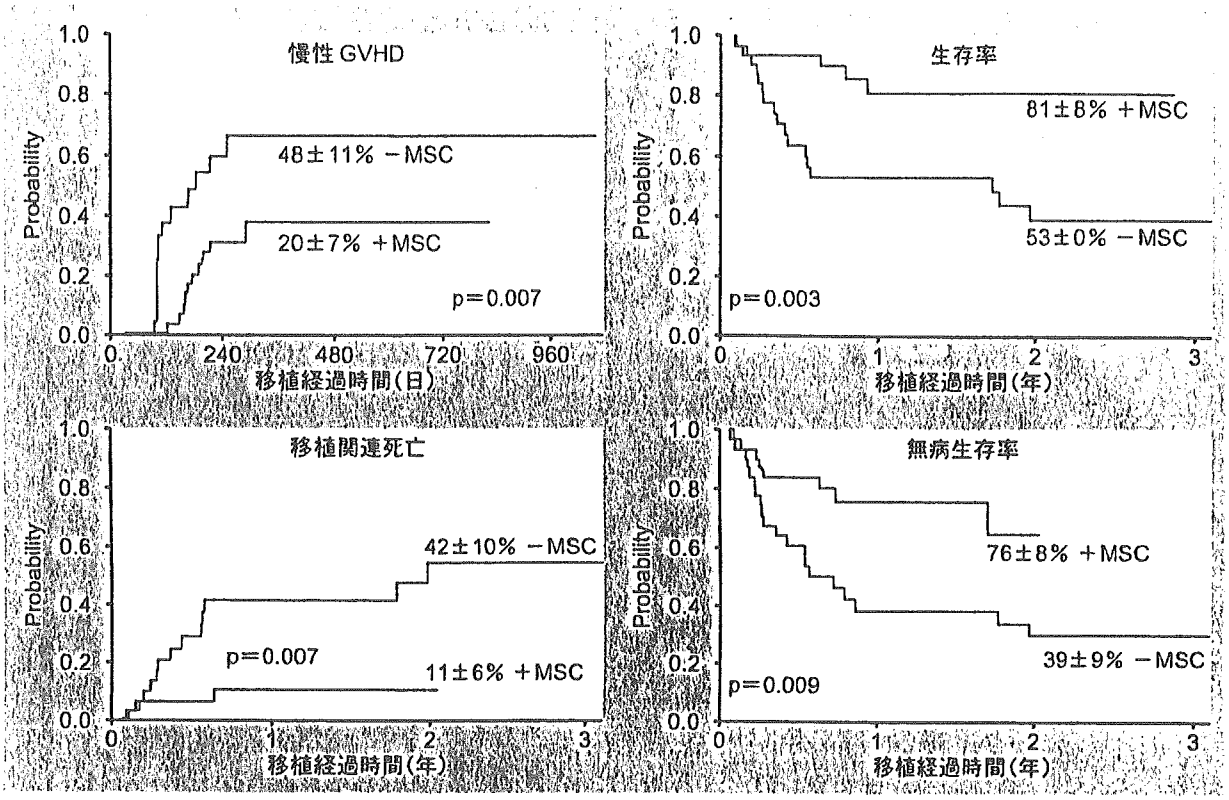

図5　GVHD，移植関連死亡，生存率に及ぼす MSC の効果

ドナーとレシピエント以外の第3者の骨髄から得られた MSC を，移植骨髄と同時に投与することによって，慢性 GVHD と移植関連死亡は有意に低下し，生存率は有意に増加した。（Osiris 資料）

対白血病効果（graft-versus-leukemia effect：GVL effect）を誘導する目的で cyclosporin を減量したところ，再度 GVHD が出現した。そこで，体重当たり 1×10^6 個の母親の MSC を再度投与したところ GVHD は終息した。MSC の投与に伴う副作用を認めなかった。著者らの施設では，Ⅳ度の急性 GVHD が出現した 25 人中で MSC を投与した本例のみが，唯一の生存例であった。

おわりに

MSC を用いた同種免疫の制御は，現在日常の臨床で行われている薬物を用いたそれに匹敵する効果が期待される。MSC 自身の抗原性が低いことから第3者（非自己および非ドナー）の MSC を用いることが出来ること，MSC 投与に伴う重篤な副作用が報告されていないことは，MSC を臨床応用するに当り大きな利点と思われる。MSC は多分化能を有するため，異所性の分化が生じる可能性は否定できないが，現在までに行われた臨床試験でそのような報告はない。今後，本邦においても造血幹細胞移植時の生着促進，GVHD 予防や GVHD 治療に対して，MSC の有用性を検討する臨床試験が早期に実施されることが期待されている。

《略語一覧》
GVL effect（graft-versus-leukemia effect；移植片対白血病効果）