Ⅴ．研究成果の刊行に関する一覧表

研究成果の刊行に関する一覧表(英文)

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VI. 研究成果の刊行物・印刷物

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Successful autologous peripheral blood stem cell transplantation with a double-conditioning regimen for recurrent hepatoblastoma after liver transplantation

Niwa A, Umeda K, Awaya T, Yui Y, Matsubara H, Hiramatsu H, Watanabe K-I, Adachi S, Itoh T, Uemoto S, Nakahata T. Successful autologous peripheral blood stem cell transplantation with a double-conditioning regimen for recurrent hepatoblastoma after liver transplantation.
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Abstract: A four-yr-old boy developed a solitary metastasis nine months after living-related liver transplantation for unresectable hepatoblastoma. After resection of the metastatic lesion, he received an auto-PBSCT with a double-conditioning regimen consisting of melphalan and thiotepa. Auto-PBSCT could be safely performed without any serious regimen-related toxicity or infection. However, transient cessation of tacrolimus during myelosuppression resulted in graft rejection of the liver just after hematological engraftment, but rejection was resolved by tacrolimus and methylprednisolone. The patient is alive and free from disease two yr after auto-PBSCT without any signs of graft rejection. High-dose chemotherapy using this conditioning regimen may be feasible for recurrent hepatoblastoma after liver transplantation in terms of safety and anti-tumor activity.

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Key words: hepatoblastoma – high-dose chemotherapy – double-conditioning regimen – liver transplantation

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Hepatoblastoma is a common malignant tumor in childhood. Combination of multidrug chemotherapy and surgical resection has improved the survival rates up to 70% (1, 2). Furthermore, LT has recently contributed to the elevation of cure rates for patients with an unresectable tumor. The prognosis of relapse cases after LT, however, remains dismal and the treatment of such cases has not been established (3, 4). Here, we report a case that underwent auto-PBSCT with high-dose chemotherapy for recurrent hepatoblastoma after LT and discuss the role of high-dose chemotherapy for such cases.

Abbreviations: AFP, alpha-fetoprotein; ALP, alkaline phosphatase; ALT, alanine transaminase; AST, aspartate transaminase; auto-PBSCT, autologous peripheral blood stem cell transplant; CBDCA, carboplatin; CDDP, cisplatin; CMV, cytomegalovirus; CT, computer tomography; EBV, Epstein–Barr virus; G-CSF, granulocyte-stimulating factor; GGT, gamma glutamyl transferase; HLA, human leukocyte antigen; IFO, ifosfamide; JPLT, Japanese Study Group for Pediatric Liver Tumor; LDH, lactate dehydrogenase; L-PAM, melpharan; LRLT, living-related liver transplantation; LT, liver transplantation; mPSL, methylprednisolone; PBSC, peripheral blood stem cell; TEPA, thiotepa; THP-ADR, tetrahydropyranyl-adriamycin; VOD, veno-occlusive disease.

Case report

A four-yr-old boy with an abdominal mass was diagnosed with embryonic hepatoblastoma by liver biopsy. At the time of diagnosis, the AFP level was 1 880 000 ng/mL. Abdominal CT showed that the tumor involved both lobes, which was categorized as pretreatment extent of disease system (PRETEXT) III (5). There was no metastatic disease detected by bone scintigraphy or chest CT scan. He was treated with two courses of CDDP 80 mg/m^2 on day one and THP-ADR 30 mg/m^2 on days one and two, and three courses of IFO 3 g/m^2 on days one and two, CBDCA 400 mg/m^2 on day three,

THP-ADR 30 mg/m^2 on days four and five, and etoposide (VP16) 100 mg/m^2 on days 1–5, according to the JPLT protocol (6), and with additional two courses of irinotecan (CPT-11) 20 mg/m^2 daily for five days. Even after those therapies, however, the size of the tumor did not change, and the AFP level remained high at 170 000 ng/mL.

At the age of four yr and eight months, the patient was transferred to our hospital for treatment of unresectable hepatoblastoma. He underwent LRLT from his mother and was treated with three courses of CPT-11 at 20 mg/m^2 daily for five days post-operatively. The AFP level normalized, and abdominal and chest CT scans showed no evidence of disease. He received tacrolimus orally and there was no sign of graft rejection.

Nine months after LRLT, the AFP levels increased to 68 ng/mL, and chest CT demonstrated a solitary tumor measuring 6.5 mm in the upper lobe of left lung. He underwent a wedge resection of left lung and histological examination of the tumor confirmed relapse of the disease. As the tumor is thought to be at least partially resistant to the standard chemotherapy used prior to LT, we planned to use L-PAM and TEPA with stem cell rescue, both of which had not been used and were expected to retain anticancer effect. Thereafter, PBSC containing 5.1 × 10^6 cells/kg CD34$^+$ cells were harvested after mobilization with nogitecan at 1 mg/m^2 daily for five days and G-CSF.

The clinical course of auto-PBSCT is shown in Fig. 1. For fear of a higher risk of severe regimen-related toxicity, the patient received a modified double-conditioning regimen (two cycles of drug combinations with a one-wk interval) that was originally reported by Hara et al. (7). The regimen consisted of 50 mg/m^2 L-PAM on days 11, 10, four, and three; TEPA of 150 mg/m^2 on days 11 and 10 and 200 mg/m^2 on days four and three. He also received ursodeoxycolic acid, low-molecular-weight heparin, antithrombin III for prophylaxis for VOD of the liver. Tacrolimus was discontinued just before the start of conditioning for fear of severe renal toxicity because of concomitant administration of L-PAM. PBSC containing 3.3 × 10^6/kg CD34$^+$ cells were infused, and G-CSF was started from day six until engraftment.

Hematological engraftment was prompt; absolute neutrophil counts reached more than 0.5 × 10^9/L on day 10; reticulocytes were more than 10‰ on day 14; platelet counts were more than 5.0 × 10^9/L on day 10. There were no severe regimen-related toxicities, such as mucositis, renal toxicity, or VOD, and no severe infections.

Pyrexia occurred on day four, and was diagnosed as clinical engraftment syndrome. The patient was treated with mPSL intravenously for four days starting on day five, which improved the symptom. Although tacrolimus was resumed on day 11, marked elevation of serum AST (477 IU/L), ALT (452 IU/L), LDH (654 IU/L), ALP (831 IU/L), and GGT (187 IU/L) levels occurred on the same day. Abdominal CT scan showed normal findings and there was no reactivation of CMV or EBV. He was diagnosed as having mild acute cellular rejection based on histological examination of a liver biopsy specimen (Fig. 2). mPSL was given intravenously for 10 days starting on day 11, and liver dysfunction rapidly improved. The patient was alive and free from disease two yr after the auto-PBSCT with no signs of graft rejection.

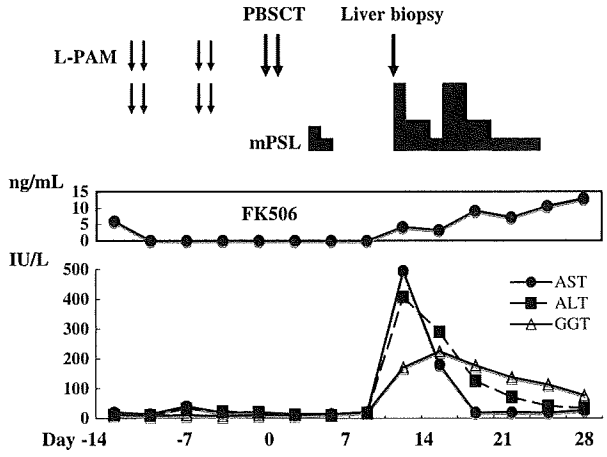

Fig. 1. The clinical course of auto-PBSCT after LT.

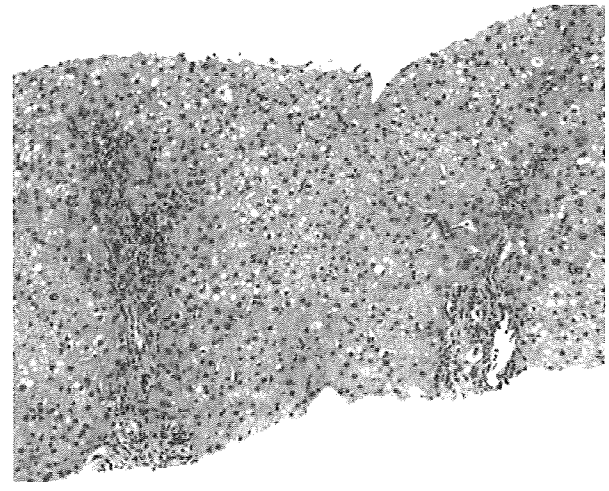

Fig. 2. Histology of liver biopsy on day 11 showing a predominantly lymphocyte infiltration in portal tracts, which was diagnosed as acute cellular graft rejection.

Discussion

Chemotherapy has improved the prognosis of hepatoblastoma (1, 2). However, the outcome of chemoresistant cases remains extremely poor, and effective treatment for such cases has not been established. Although the role of high-dose chemotherapy in the treatment of hepatoblastoma remains controversial, there are some reports describing auto-PBSCT for hepatoblastoma (7–9). Our case underwent LRLT but a solitary lung metastasis developed. Although the metastatic lesion could be removed completely, the prognosis was thought to be poor, since previous reports indicated that most patients with relapse after LT died their disease (4). Moreover, these cases were thought to be resistant to CDDP, THP-ADR, IFO, CBDCA, and VP16 at standard doses, which are the key drugs to treat hepatoblastoma. Therefore, we used high-dose chemotherapy with stem cell rescue using two alkylating agents, L-PAM and TEPA, which had not been used in this case and are reported to have potent anti-tumor activity (7).

There has not been any report of successful auto-PBSCT after LT. Compared with conventional auto-PBSCT, it remains unknown whether the preconditioning regimen could be tolerated by patients with a transplanted liver. Double-conditioning regimen (two cycles of drug administration with a one-wk interval), a modification of the treatment reported by Hara et al. (7), was selected to reduce regimen-related toxicities to a minimum while retaining the anti-cancer effect. Indeed, there was no serious regimen-related toxicity in this case. There was no severe infection either, despite using immunosuppressive agents to prevent graft rejection after LT. Although the follow-up period remains relatively short, high-dose chemotherapy with auto-PBSCT might be effective as a consolidation therapy after resection of a metastatic lesion. Thus, high-dose chemotherapy with stem cell rescue could be considered for cases with metastatic or relapsed tumors that were resistant to the standard chemotherapy.

Even in conventional auto-PBSCT, the combination of L-PAM and TEPA has been reported to cause severe renal toxicity (7). To reduce the risk of severe renal toxicity, the total dose of L-PAM was reduced from 280 mg/m^2, that was originally reported (7), to 200 mg/m^2. Furthermore, to gain sufficient time for the elimination of L-PAM, tacrolimus was discontinued from the beginning of the conditioning regimen until hematological engraftment. As a result, graft rejection did occur on day 11 although it had not previously been observed before auto-PBSCT. In cases undergoing allogeneic BMT from HLA-matched sibling donor after LT, graft rejection was not reported when immunosuppressive agents were transiently discontinued during administration of preconditioning drugs, then restarted from one day before transplantation (10, 11). Therefore, it is suggested that at the early stage of auto-PBSCT, only a few engrafted cells can cause graft rejection in the absence of immunosuppressive agents. Low dose of tacrolimus or steroids after conditioning might reduce the risk of graft rejection.

In conclusion, we successfully performed auto-PBSCT after the double-conditioning regimen with L-PAM and TEPA for recurrent hepatoblastoma after LT. If the patient is in good condition, the conditioning regimen with L-PAM and TEPA can be performed safely after LT, and possibly prevent relapse of hepatoblastoma that is refractory against standard chemotherapies. More sophisticated immunosuppressive therapy after conditioning will be required to prevent graft rejection.

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ORIGINAL ARTICLE

AKR-501 (YM477) a novel orally-active thrombopoietin receptor agonist

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Abstract

Thrombopoietin (TPO) is the principal physiologic regulator of platelet production. We have searched for small molecule compounds that mimic the action of TPO by using human TPO receptor-expressed in Ba/F3 cells, resulting in the discovery of AKR-501 (YM477). AKR-501 specifically targeted the TPO receptor and stimulated megakaryocytopoiesis throughout the development and maturation of megakaryocytes just as rhTPO did. AKR-501, however, was shown to be effective only in humans and chimpanzees with high species specificity. Therefore, we examined the *in vivo* platelet-increasing effect of AKR-501 in human platelet producing non-obese diabetic/severe combined immunodeficiency (NOD/SCID) mice transplanted with human fetal liver $CD34^+$ cells. Daily oral administration of AKR-501 dose-dependently increased the number of human platelets in these mice, with significance achieved at doses of 1 mg/kg and above. The peak unbound plasma concentrations of AKR-501 after administration at 1 mg/kg in NOD/SCID mice were similar to those observed following administration of an active oral dose in human subjects. These results suggest that AKR-501 is an orally-active TPO receptor agonist that may be useful in the treatment of patients with thrombocytopenia.

Key words thrombopoietin; thrombopoiesis; c-mpl; platelets; NOD/SCID mice; AKR-501; YM477

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While platelet development appears to be regulated by a number of growth factors and cytokines, thrombopoietin (TPO) is the principal physiologic regulator of platelet production (1, 2). TPO stimulates megakaryocytopoiesis throughout the development and maturation of megakaryocytes leading to platelet production. The TPO receptor is expressed on hematopoietic stem cells (HSCs), a subfraction of hematopoietic precursors, and on cells of the megakaryocytic lineage and platelets. Two TPO receptor agonists, recombinant human TPO (rhTPO) and pegylated recombinant megakaryocyte growth and development factor (PEG-rHuMGDF), have previously undergone extensive clinical trials. However, the clinical development of PEG-rHuMGDF was terminated because of the development of neutralizing antibodies to PEG-rHuMGDF that cross-reacted with endogenous thrombopoietin, resulting in profound hematological consequences in patients and volunteers receiving multiple subcutaneous injections of the drug (3, 4). The appearance of antibodies was reported in an early trial of rhTPO, but the biological activity of these antibodies has not been reported (5). Recombinant

TPOs are therefore associated with risk of immunogenicity.

Recently, a number of screening methods have been developed to identify small molecules that could mimic the biological effects of hematopoietic growth factors. Potential advantages of small molecule mimetics include their putative lack of immunogenicity and non-parenteral route of administration. Small molecules might also be less expensive to produce. To identify small molecule mimetics of native proteins, high throughput assays of either receptor binding or biological activity have been devised. With respect to TPO, several small molecular compounds were reported to mimic the effect of TPO through the TPO receptor (6–10). Therefore, we screened by using human TPO receptor transfected murine Ba/F3 cells to identify orally active small molecular TPO receptor agonists (11), which led to the discovery of AKR-501.

Here, we describe the pharmacological properties of AKR-501. We showed that AKR-501 mimics the biological activities of TPO *in vitro* and *in vivo*. AKR-501, however, was showed to have effect in humans and chimpanzees only. Non-obese diabetic/severe combined immunodeficiency (NOD/SCID) mice were characterized as representing an efficient engraftment model for human HSCs, which resulted in the production of human platelets. Therefore, this model was used in this study to examine the effect of AKR-501 on *in vivo* platelet production in human platelet producing NOD/SCID mice in which human HSCs has been transplanted. Further, we demonstrated that this model is suitable to predict the effect of AKR-501 in humans.

Materials and methods

Compounds

AKR-501, 1-(3-Chloro-5-{[4-(4-chloro-2-thienyl)-5-(4-cyclohexylpiperazin-1-yl)-1,3-thiazol-2-yl]carbamoyl}-2-pyridyl) piperidine-4-carboxylic acid was synthesized by Yamanouchi Pharmaceutical Co., Ltd. (Ibaraki, Japan) (12). rhTPO was purchased from GT (Minneapolis, MN, USA).

Human TPO receptor expressed Ba/F3 cells

The human full-length *c-mpl* cDNA (*c-mpl-p*) derived from HEL cells was subcloned into a pEF-BOS vector. This plasmid pEF-BOS-*c-mpl* (10 μg) and the selection marker plasmid pSV2bsr (1 μg) (Kaken-Seiyaku, Tokyo, Japan) were co-electroporated into murine IL-3 (mIL-3) dependent murine Ba/F3 cells in a 0.4 cm cuvette at 1.5 kV (25 μF). These cells were then cultured in RPMI 1640 medium (GIBCO, Grand Island, NY, USA) supplemented with WEHI conditioned media (BD Biosciences, Bedford, MA, USA) for 3 d, and then in selection medium containing 10 μg/mL blasticidin (Funakoshi, Tokyo, Japan) for 1 month.

Assay for TPO receptor-dependent cell growth

The cells used in this study were maintained in RPMI 1640 medium supplemented with 10% FBS (JRH BIOSCIENCES, Lenexa, KS, USA), 50 units/mL penicillin/streptomycin (GIBCO), and WEHI conditioned media. These cells were seeded in each well of a 96-well plate and cultured in the medium at the concentration of 2×10^5 cells/mL. Human TPO receptor expressing Ba/F3 cells were incubated with AKR-501, dissolved in dimethyl sulfoxide (DMSO), or rhTPO. Ba/F3 cells were incubated with AKR-501, rhTPO or recombinant mIL-3 (rmIL-3; Pepro Tech EC, London, UK). WST-1/1-methoxy PMS reagent (Cell counting kit, Dojin, Kumamoto, Japan) was added to each well and then incubated. A450/A655 was measured with a microplate reader (Model 3350; BioRad, Hercules, CA, USA) immediately and at 2 h after addition of WST-1/1-methoxy PMS reagent. Proliferation activity of the compound was calculated as the percentage of maximum proliferative activity of rhTPO for TPO receptor expressing Ba/F3 cells. The percentage of maximum proliferative activity of rmIL-3 was used to calculate the proliferation activity for Ba/F3 cells.

Megakaryocyte colony formation

Human cord blood (CB) $CD34^+$ cells (AllCells, Berkeley, CA, USA) at a density of 2500 cells/0.75 mL were suspended in MegaCult™-C (StemCell Technologies Inc., Vancouver, Canada) containing either AKR-501 dissolved in DMSO or rhTPO, and plated in two-well chamber slides. After 10–12 d of incubation, human CD41-positive megakaryocytes were identified. Colonies that contained three or more human CD41-positive cells were scored as human megakaryocyte colonies. Data were expressed as the percentage of maximum differentiation activity of rhTPO, and the EC_{50} values were calculated for the drug.

Ploidy analysis

G-CSF-mobilized human peripheral blood $CD34^+$ cells (BioWhittaker Inc., Walkersville, MD, USA) were seeded in a 24-well culture plate (Iwaki, Chiba, Japan) at 10 000 cells/0.5 mL/well in culture medium in the presence of 3 μM AKR-501 or 3 nM rhTPO. The culture medium consisted of 100 μM 2-mercaptoethanol, 7.5 μg/mL