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Acute radiation syndrome: On the issues of triage, stem cell sources and immunosuppressive therapy

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Case Report

Keywords: Acute radiation syndrome (ARS), triage, hematopoietic stem cell transplantation (HSCT), umbilical cord blood (UCB)

Posted Date: May 16th, 2022

DOI: https://doi.org/10.21203/rs.3.rs-1648490/v1

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Abstract

We treated the highly gamma- and neutron-irradiated nuclear accident victim by the criticality fission reaction. Conventional dosimetry methods posed us the risk of a treatment delay, and later established organ-oriented dose estimation METREPOL criteria is a helpful tool for rapid triage and stratification of the acute radiation syndrome (ARS) patients. Graft-versus-host disease (GVHD) is the most predominant life-threatening factor for ARS. Umbilical cord blood (UCB) provides us the rapid availability during the golden time for hematopoietic stem cell transplantation (HSCT) with lower risk of GVHD. Current advances of supportive care provide us the safer HSCT, so UCB overweigh the risk of engraftment failure and delayed hematopoietic recovery compared to the matched unrelated donor or related one haploidentical donor. UCB is an ideal stem cell source for HSCT of ARS, if the patients lack the suitable donor candidate.

Introduction

Recent global situations, including international conflicts and terrorist attacks, have revealed the threat of nuclear events and the necessity of preparedness. We previously described the case of a highly gamma- and neutron-irradiated victim of a criticality nuclear accident[1]. We reassessed our intervention and we call for the discussion of an expertise specialists.

Results

Dosimetry

At that time, dosimetry was based on the hemogram (mainly by lymphocytes and platelets counts), chromosomal analysis, and serial measurement of the attenuation of emitted intrabody ^{24}Na with a phantom reconstitution. The final estimated dose of whole-body irradiation was 9–10 GyEq, and repeated sternal and iliac bone marrow aspirations indicated marked bone marrow hypoplasia with scarce cellularity. Autologous hematopoietic recovery was unlikely. The International Atomic Energy Agency (IAEA) published the treatment guideline “Diagnosis and treatment of radiation injuries”. Based on this guideline, the indication for hematopoietic stem cell transplantation (HSCT) is clearly defined as follows: “HSCT should be considered only for victims receiving doses in the range of 8–12 Gy, uniformly distributed, without serious skin injuries, and in the absence of severe internal contamination and conventional injuries. The timing of grafting is important, and all arguments favour early marrow transplantation, even within the first week after exposure”. Our victim met these descriptions.

Stem cell source

He lacked a suitable HLA-matched sibling donor (MSD), and it would take a median of six months for donor coordination in the Japan Marrow Donor Program. To avoid missing the critical window for HSCT, we decided to transplant umbilical cord blood (UCB) from an unrelated female donor with seven of eight matching HLA loci (DRB1-mismatched).

Pre-transplant conditioning

After non-myelotoxic conditioning consisting of 5 mg/kg of anti-thymocyte equine gamma globulin (ATG) alone (almost half of the conventional dose; in addition, we avoided cytotoxic drugs given the ensuing mucositis), a total of 2.08 x 10^7 UCB mononuclear cells (MNCs) ($CD34^+$ cells dose: 7.11 x 10^4/kg) were infused nine days after irradiation.

Graft-versus-host disease (GVHD) prophylaxis

Graft-versus-host disease prophylaxis consisted of intravenous (i.v.) cyclosporine (CyA) 3 mg/kg with i.v. methylprednisolone (mPSL) 4 mg/kg and then gradually tapering. To augment the hematopoietic recovery, we concurrently administered granulocyte colony stimulating factor (G-CSF; 5 mg/kg), erythropoietin (EPO; 100 IU/kg) and thrombopoietin (TPO; 5 mg/kg) as compassionate care. His neutrophil count reached 0.5 x 10^9/L on day 15, his reticulocyte count rose above 1% of the total red blood cell count on day 23 and his platelet count reached 50 x 10^9/L on day 27. Fluorescence *in situ* hybridization using a Y-chromosome-specific probe (Y-probe FISH) indicated that almost 60% of sternal bone marrow MNCs and 40% of iliac bone marrow MNCs were donor derived on day 9 after UCB transplantation (UCBT), and 50% of peripheral blood MNCs on day 13 showed donor-derived XX signals. After obtaining mixed chimerism, we gradually tapered CyA and mPSL to avoid infection and to augment autologous hematopoietic recovery. Donor-derived MNCs representing XX signals completely disappeared at 50 days after UCBT. Although repeated cutaneous biopsy on day 24, day 40, and day 94 indicated no evidence of GVHD, the patient's deep dermal neutron burns markedly progressed to 67% of the body surface area on his systemic skin. As a cutaneous infection preventative measure, we performed cadaver skin transplantation on both arms on day 72, both legs on day 80 and on his face on day 114 after UCBT. Despite our effort, the patient developed a cutaneous vancomycin-resistant *Staphylococcus aureus* (VRSA) infection and oral stomatitis due to ARS and finally succumbed from aspiration pneumonia with acute respiratory distress syndrome (ARDS) on day 201 post transplantation (210 days after lethal irradiation).

Discussion

Tokaimura's experience convinced us that the triage would be delayed if we depended on conventional dosimetry. Based on these experiences, Fliedner TM *et al*. established practical triage methods according to an organ-oriented symptom-guided grading system, named METREPOL[2]. Symptoms of acute radiation syndrome (ARS) are evaluated in each radiosensitive organ according to severity. Damage to the neurological system (Ni), hematological system (Hi), cutaneous system (Ci) and gastrointestinal (GI) system (Gi) are graded for each organ. Based on these criteria, on day 7, our victim was N2 H4 C3 G1, RC = 4_{d7}. RC4 patients should be considered for transfer to a highly equipped hospital for supportive care or HSCT. The promotion of METREPOL information among all health-care providers is an urgent task in the current worldwide situations.

Waselenko JK *et al*. recommended in their clinical guidelines of the Strategic National Stockpile Radiation Working Group that HLA-matched related and unrelated allogeneic stem cell transplantations are lifesaving and potentially curative treatments in patients with certain predominantly hematological malignant conditions[3]. In the Chernobyl experience, thirteen patients who were exposed to an estimated whole-body dose of 5.6 to 13.4 Gy received bone marrow transplants[4]. Nine recipients showed hematopoietic recovery; six out of these nine recipients showed transient partial engraftment, and GVHD were diagnosed in four and suspected in two other recipients. Densow D *et al*. reviewed the cases of 4,500 victims, significantly exposed in 450 radiation accidents worldwide. Among them, twenty-nine recipients received allogeneic bone marrow or fetal liver cells (FLCs), but all patients with burns died, and only three out of 29 lived beyond one year[5]. Four years later, the US National Marrow Donor Program (NMDP) and American Society for Blood and Marrow Transplantation established the Radiation Injury Treatment Network (RITN)[6]. Based on the results of thirty-one HSCT recipients, the median survival for these patients was approximately one month, and all four recipients who survived more than one year showed autologous hematopoietic recovery. The RITN also raised the question of whether HSCT provides any benefit; in particular, more than 20% of recipients died from GVHD. These findings indicated that the avoidance of GVHD is the most critical problem in HSCT for ARS. There are several possibilities for donor candidates and stem cell sources for HSCT for ARS (**Figure**).

Considering the engraftment rate and risk of infection/GVHD, an MSD is the highest donor candidate priority. (1) If the victim lacks an MSD, considering the rapid availability of UCB during the HSCT critical window, UCBT has a significant advantage compared to matched unrelated donor (MUD) and related one-haploidentical donor (Haplo) transplantation. MUD transplantation requires several months for the coordination and confirmation of donor eligibility, and Haplo transplantation requires growth factor administration to donors for mobilization and harvesting. Cryopreserved and pathogen-tested UCB is a ready-to-use stem cell source of HSCs for patients with ARS. (2) MUD transplantation offers the best possibility of engraftment, as there is a limited stem cell dose in UCBT and HLA disparity in the Haplo setting. (3) Considering the risk of infection, UCBT requires a prolonged time for hematopoietic recovery, and Haplo transplantation requires sustained immunosuppressive therapy (IST). As described below, the low risk of GVHD in the UCBT setting permits the early discontinuation of IST, but UCBT has a concomitant risk of graft rejection, as in our patient. However, recent advances in clinical pharmacology have provided us with more effective novel antibiotics, antiviral drugs (e.g., letermovir) and antifungal medication (e.g., posaconazole). The indications for HSCT have been significantly widened compared to our previous experience 20 years ago. (4) The most critical issues in the treatment of ARS are the avoidance of GVHD and the worsening of cutaneous/GI syndromes. The current regimen is one-haploidentical HSCT using post-transplantation cyclophosphamide (CY). ARS causes severe damage to the exposed skin and GI mucosa. Endothelial damage is an ARS worsening factor. MUD transplantation involves a potential risk of GVHD that inadvertently worsens ARS. Furthermore, post-transplantation CY inevitably complicates regimen-related toxicities (RRTs) involving the GI mucosa and hematopoiesis. Considering the risk of RRT, CY may be one of the contraindicated drugs for ARS. In our patient who underwent UCBT, we showed that there was no evidence of cutaneous GVHD (on days 24, 40, 94) or gut

GVHD (on day 40) by repeated biopsy[1]. We have previously described that UCB T cells have an impaired response to CD3/CD28 stimulation with decreased cell surface expression of costimulatory CD40L and FasL. These results indicate the bipotent risk and benefit of UCB T cells, as they are associated with a decreased risk of GVHD but an increased risk of infection[7]. The current understanding of stem cell biology clearly documents that HSCs have a definite fate of aging in a murine model. Aged HSCs exhibit functional alterations, including a loss of immune function and reduced regenerative capacity and myeloid-biased differentiation. Rossi *et al*. highly purified murine c-kit^{+}lin^{-}Sca-1^{+}flk2^{-}CD34^{-} long-term HSCs (LT-HSCs), and they demonstrated the contribution of these cells to adult impaired lymphopoiesis[8]. Other mechanisms, including accumulated DNA damage, impaired levels of autophagy, accumulated metabolic stress with DNA mutations in mitochondria, and epigenetic reprogramming with aged clonal hematopoiesis, concurrently affect the self-renewal properties of aged HSCs[9]. In human patients, Vaziri *et al*. clearly documented the shortened telomeric DNA of human bone marrow HSCs compared to UCB-derived HSCs[10]. Shortened telomeric DNA strongly suggests the limited proliferating potential of bone marrow HSCs compared to UCB HSCs. Furthermore, impaired cutaneous re-epithelization and GI mucosa regeneration stem from impaired endothelial regeneration. We have previously described that lineage-negative (Lin^{-}) CD34^{+} cells in UCB expressed TIE2 (18.8%)[11]. TIE2 is a marker of HSCs in UCB, and the TIE2 ligand Ang-1 promotes HSC adhesion to fibronectin and the maintenance of HSC stemness. These results indicate that human UCB contains bipotent Lin^{-}CD34^{+}TIE2^{+} hematopoietic and endothelial progenitors with the potential contribution of HSCs for endothelial regeneration in UCBT in ARS settings.

A severely irradiated cell expresses mutated DNA transcripts and aberrant proteins on its cell surface. These molecules may be candidate epitopes for autologous immune system targets. GVHD is an allogeneic immune reaction initially evoked by a pretransplant conditioning regimen consisting of high-dose chemoradiation ensuing from tissue damage[12]. GVHD causes the release of pathogen-associated molecular patterns (PAMPs) and various chemokines and cytokines (TNF-a, IL-1b, IL-6, IFN-g, etc.). Finally, host- and donor-derived antigen-presenting cells (APCs) activate donor-acquired immune cells, including type 1 and type 17 CD4^{+} helper cells (Th1 and Th17 cells, respectively), type 1 CD8^{+} cytotoxic T lymphocytes (Tc1 cells), natural killer (NK) cells, macrophages and B cells. These reactions markedly resemble the pathophysiology of ARS. Appropriate IST is mandatory to overcome ARS. Recent advances in IST provide several treatment options for GVHD. Not only conventional IST drugs (calcineurin inhibitors, mTOR inhibitors, antimetabolites, and corticosteroids) but also tyrosine kinase inhibitors and several monoclonal antibodies targeting T cells or cytokines lead to clinical responses in patients with a steroid-resistant GVHD.

If there are no suitable donor candidates, cryopreserved UCB is a promising source of HSCs for the treatment of ARS. The rapid availability of UCB and limited risk of GVHD with UCBT are advantages of UCBT compared with MUD or Haplo transplantation, considering the risk of engraftment failure and susceptibility to infection. Supportive care in HSCT has markedly progressed and includes the possible use of growth factors (keratinocyte growth factor, KGF), more feasible and effective antibiotics, and antiviral/antifungal agents with effective IST. A high-quality UCB-banking system is mandatory for

preparedness against nuclear events. Further discussion of triage, indication or donors for HSCT and suitable IST for ARS is necessary for future victims.

Methods

Patient

The criticality nuclear fission was incidentally provoked during the uranium (UF_6) concentrating process at the nuclear facility at JCO, Tokaimura, Japan, on September 30th, 1999. Two untrained parttime workers were lethally irradiated, and one of the two 39-year-old male victim was transferred to our hospital for hematopoietic rescue.

Patient material

Patient materials including peripheral blood and bone marrow aspiration samples, cutaneous and gastrointestinal mucosa biopsy materials are all corrected after signed informed consent according to the Declaration of Helsinki and used after patient's permissions.

Triage and treatment

All triage and treatment were based on the guideline distributed by International Atomic Energy Agencies (IAEA) Safety Reports Series No.2 "Diagnosis and Treatment of Radiation Injuries" (IAEA, Vienna 1998). IAEA inspects the treatment and transplantation. IAEA sends the advisory committee consists of Dr. Alexander Baranov (Russia), Dr. Fred A. Metler (United States), Dr. Theodor M. Fliedner (Germany), Dr. Norbert-Claude Gorin and Patrick Goumelon (France). Nuclear Safety Commission (NSC) of Japan cabinet and Institutional Review Board (IRB) of The Institute of Medical Science, The University of Tokyo (IMSUT) approved the decision of IAEA advisory.

Declarations

Acknowledgments

We thank Professor Makoto Akashi (Tokyo Health Care University) and Kazuhiko Maekawa (ex. The University of Tokyo) for his critical comments and instruction of clinical treatment.

HN thank for Prof. Guojun Sheng, Prof. Hitoshi Takizawa, and Director Toshio Suda for their organization and Noriko Tokushige, Junko Kurowarabi, and Hanami Sakai for their secretary assistance for IRCMS.

Author contributions

HN and JO, both examined, transplanted, and cared the victim. HN and JO described the manuscript and HN made the Figure 1.

Data availability

All data analyzed during this study are included in these published articles. (Supplementary results)

1. Transplantation of the case (Ref.1)

Nagayama, H. *et al.* Transient hematopoietic stem cell rescue using umbilical cord blood for a lethally irradiated nuclear accident victim. *Bone Marrow Transplant* **29**, 197-204, doi:10.1038/sj.bmt.1703356 (2002).

2. Clinical course after transplantation

Nagayama, H., Ooi J., *et al.* Severe immune dysfunction after lethal neutron irradiation in a JCO nuclear facility accident victim. *Int. J. Hematol*. **76**: 157-164, doi: 10.1007/BF02982579 (2002).

3. T cell profile of umbilical cord blood (UCB) (Ref. 7)

Sato, K., Nagayama, H. & Takahashi, T. A. Aberrant CD3- and CD28-mediated signaling events in cord blood T cells are associated with dysfunctional regulation of Fas ligand-mediated cytotoxicity. *J Immunol* **162**, 4464-4471 (1999).

4. Stem cell profile of UCB (Ref. 11)

Yuasa, H. *et al.* Analysis of human TIE2 function on hematopoietic stem cells in umbilical cord blood. *Biochem Biophys Res Commun* **298**, 731-737, doi:10.1016/s0006-291x(02)02524-x (2002).

Declaration

The authors declare that they have no conflicts of interest.

All patient materials including peripheral blood or bone marrow aspirate, and cutaneous/gastrointestinal biopsy specimens were collected and used after signed informed consent according to the Declaration of Helsinki.

This treatment was done before the establishment of International Society for Stem Cell Research (ISSCR) Guideline for Stem Cell Research and Clinical Translation. But almost it is in accordance with current ISSCR guideline.

Nuclear Safety Commission (NSC) of Japan cabinet and Institutional Review Board (IRB) of The Institute of Medical Science, The University of Tokyo (IMSUT) approved the decision of IAEA advisory.

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Figures

Figure 1

	MUD	UCB	Haplo
Rapid availability	Need coordinate	**Immediate**	Need harvest
Success of engraftment	High	Low	Low
Risk of infections	Moderate	High	High (IST)
Risk of GVHD	High	**Minimal**	Very high

Figure 1

Donor candidates for hematopoietic stem cell transplantation (HSCT) for ARS patients

ARS: acute radiation syndrome, MUD: HLA-**m**atched **u**nrelated **d**onor, UCB: **u**mbilical **c**ord blood, Haplo: One-**haplo**identical related donor, GVHD: graft-versus-host disease, IST: immunosuppressive therapy

Supplementary Files

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