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Case report

Transplantation for accidental acute high-dose total body neutron- and γ-radiation exposure

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Summary:

Accidental exposure to acute high-dose total body neutron radiation is rare. We report a 35-year-old man exposed to a total body dose of 5.4 Gy neutron- and 8.5–13 Gy γ-radiation in a radiation criticality accident. He received a blood stem cell transplant from his HLA-identical sister. There was bone marrow recovery with complete donor chimerism. Random chromatid breaks were observed in donor cells suggesting a bystander effect of neutron exposure. The subject died 82 days after the accident (75 days post transplant) from multi-organ failure.

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Keywords: transplantation; radiation; neutron; γ-ray; bystander effect

Accidental exposure to acute high-dose total body neutron radiation is rare; only six victims have been reported previously.[1] The main targets of damage in this setting are the bone marrow, gastrointestinal tract, lungs and brain.[2–4] Bone marrow and fetal liver transplants have been used to treat 29 victims of radiation accidents since 1958.[5–10] Most of these accident victims received acute high-dose γ-radiation. We used concepts developed from these data to treat a man exposed to acute high-dose neutron- and γ-radiation with a blood stem cell transplant from his HLA-identical sister.

Case report

A radiation criticality accident occurred on 30 September 1999 in Tokaimura, Ibaraki-ken, Japan at a nuclear fuel processing plant when two workers prepared excess uranyl nitrate in a precipitation tank. One victim, described in this report, saw 'blue light' (Cherenkov reaction) and evacuated immediately to a room 25 m from the scene where he lost consciousness for 1 min and vomited within 10 min. Diarrhea developed within 70 min and continued for 2 days. One hundred minutes after exposure, the WBC was 23×10^9/l and blood lymphocytes were 0.7×10^9/l. Biological dosimetry estimated from the rate of decline of WBC and lymphocytes, was >10 Gy.[11] Cytogenetic studies were consistent with a dose of >20 Gy.[12] Physical dosimetry using ^{24}Na capture was 5.4 Gy neutron- (RBE 1.5–2.0; 8.1–10.8 Gy equivalent) and 8.5–13 Gy γ-radiation, depending on the method of γ-dose estimation.[13]

Two days post accident, the victim was transferred to the University of Tokyo Hospital. He was alert; temperature was 37°C, blood pressure 120/70 mm Hg and pulse 90–100/min. There was severe painful erythema prominent in the right arm and moderate on the face, ventral trunk and left arm (corresponding to a first-degree thermal burn). Physical examination was otherwise normal.

WBC was 22×10^9 with no lymphocytes; hemoglobin was 13.4 g/dl and platelets, 120×10^9/l. Blood chemical tests were normal except for increased amylase (301 IU/l; normal, 66–220 IU/l) and C-reactive protein (4.5 mg/dl; normal <0.3 mg/dl).

A bone marrow aspirate and biopsy from the iliac crest 3 days post accident was 10% cellular. A simultaneous bone marrow aspirate from the sternum was <10% cellular. In both samples, most nucleated cells were mature granulocytes (75–90%; see Table 1). There were many macrophages. Few erythroblasts were seen and there were no erythroid islands. Megakaryocytes were severely reduced in number. Karyorrhexis and pyknosis were seen in some remaining granulocytes and nuclear degeneration in megakaryocytes. We identified 16 metaphases after 24 h incubation of bone marrow cells from iliac crest (11) and sternum (5), all of which had at least one dicentric chromosome. Some chromosomes had more than two centromeres. Formal karyotype analysis was impossible because of extensive chromosome breaks.

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Table 1 Bone marrow examinations

Days (PT)	*3 (PA)*	*3 (PA)*	*9*	*9*	*15*	*21*	*34*	*41*	*48*	*55*	*67*
Sites	ST	IL	ST	IL	IL[a]	IL	IL	IL	IL	IL	IL
Cellularity(%)	<10	10	50	20	20–30	40–50	30–35	30–35	20–25	20	10–15
M/E	>>10	>>10	4–5	4–5	10–15	8–12	8–10	3–4	1–1.5	0.8–1	0.2
Blasts + Pro	<1	1	3–5	5–7	8–12	6–10	6–10	1	3–5	1–2	<1
Myelo + Meta	1–2	3–5	50–55	55–60	35–40	30–35	25–30	10–15	33–38	17–22	10–15
Stab + Seg	80–90	75–85	10–15	5–10	35–40	40–45	50–55	60–70	10–15	20–25	6–10
Eosino	1	1–2	1	1–2	1	1	<1	<1	1–2	<1	1–2
Baso	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1
Erythrobl	<1	1	15–20	15–20	6–10	6–10	7–11	15–20	40–45	43–48	65–75
Mono	1–2	1	3–5	3–5	4–6	5–7	<1	2–3	<1	1–2	1
Macroϕ	6–10	6–10	1–2	1–2	1	<1	<1	<1	1	4–6	1–2
Lymph	1–2	3–5	3–5	2–3	<1	1	3–5	2–3	3–5	3–5	3–5
Plasma	1	1–2	1–2	1–2	<1	1	<1	<1	<1	<1	<1

PT = post transplant; PA = post accident; ST = sternum; IL = iliac crest.
[a]Bone marrow sampling from sternum was unsuccessful after day 9.

Because of the high estimated total body radiation dose and consequent high likelihood of severe bone marrow failure,[3,11] a transplant was considered after identifying an HLA-A, B, C and DR-identical sister. Blood cells (1.84×10^8 CD34-positive cells; 2.3×10^6/kg recipient body weight) were obtained from the donor by apheresis after treatment with granulocyte colony-stimulating factor (G-CSF) and infused into the subject on days 6 and 7 post accident (transplant day 0). Tacrolimus, continuous infusion at an initial dose of 0.02 mg/kg/day, adjusted to achieve a serum concentration of 10–15 ng/ml, and one dose of methotrexate, 15 mg/m^2, were given post transplant to prevent graft-versus-host disease (GVHD). G-CSF, 175 μ/m^2, was given post transplant from days 0 to 8 to accelerate bone marrow recovery. RBC and platelet transfusions were initiated from days 7 and 5 post transplant, respectively. Post accident course is summarized in Figure 1.

Granulocytes reached $>0.5 \times 10^9$/l 9 days post transplant. Bone marrow aspirates from both sternum and left iliac crest 9 days post transplant showed regenerating hematopoietic cells. Clot sections of the sternal and iliac bone marrow showed cellularity of 50% and 20%, respectively. Differential counts in both bone marrow samples were similar: 65–80% granulocytes (predominantly myelocytes – metamyelocytes), 3–5% monocytes, 1–2% macrophages, 2–5% lymphocytes, 1–2% plasma cells and 15–20% erythroblasts (Table 1). Erythroblast islands were rare and there were small numbers of megakaryocytes. Occasional erythrophagocytosis was seen. Cytogenetic analysis showed a donor (46, XX) karyotype in all 60 metaphases examined (30 from sternal and 30 from iliac bone marrow). Three metaphases from the sternum showed random chromosome breaks in donor cells (Figure 2). Fluorescence *in situ* hybridization (FISH) analysis showed that 589 of 600 cells (98%) were XX. A bone marrow aspirate from the iliac crest 21 days post transplant showed 40–50% cellularity and M/E ratio >10, and XY-FISH analysis showed only 1% XY cells. Similar results were obtained repeatedly in the ensuing FISH analyses. When possible, bone marrow cells were separated into CD3-positive (T cell) and negative (non-T cell) fractions and XY-FISH analysis on each population was performed. Only the T cell fraction (<10% of nucleated cells) contained XY cells. The maximum ratio of XY (recipient) to XX (donor) T cells was 0.12.

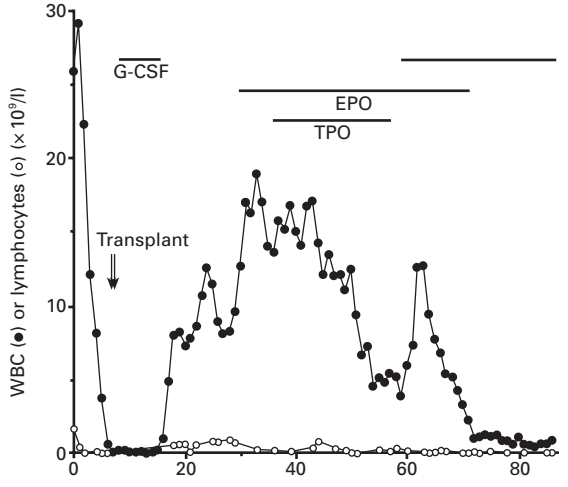

Figure 1 Post-accident course. G-CSF, granulocyte colony-stimulating factor; EPO, erythropoietin; TPO, thrombopoietin.

The WBC increased to $>10 \times 10^9$/l and remained elevated after G-CSF was discontinued. However, on day 49 post transplant the WBC decreased to 2.7×10^9/l despite restarting G-CSF and by day 65 post transplant it was $<1.0 \times 10^9$/l. A bone marrow aspirate from iliac crest 48 days post transplant showed decreased cellularity; decrease in mature granulocytes was remarkable. A bone marrow aspirate 55 days post transplant showed a marked decrease in myelopoiesis with conspicuous intermediate erythroblasts, and extensive erythrophagocytosis. Dysplastic changes such as nuclear maturation delay and impaired enucleation were evident in erythroblasts. There were few erythroblast islands and megakaryocytes. Iron deposition was striking. A bone marrow on day 67 was more hypocellular with an M/E ratio of 0.2 and persistent erythrophagocytosis. Because of persistently low levels of RBCs and platelets, erythropoietin and thrombopoietin were given with no

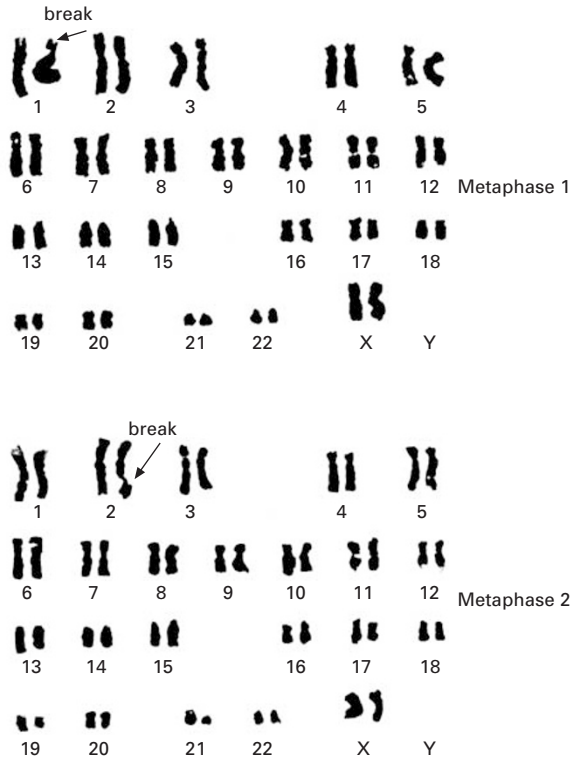

Figure 2 Metaphases in sternum bone marrow cells 9 days post transplant.

detectable effect and the subject required frequent RBC and platelet transfusions until death. Myelocytes appeared 11 days post transplant in the blood, peaked on days 21 (4%), and decreased thereafter. Erythroblasts also appeared in the blood, peaking as 20% of WBCs on day 48.

Rash, diarrhea and hyperbilirubinemia were present post transplant. Whether these were from radiation and/or acute GVHD could not be accurately determined especially in the context of corticosteroid therapy.

Fifty days post transplant there was a cardiac arrest of undetermined etiology. Although resuscitation was successful, the subject developed tissue hypoperfusion and died of multi-organ failure 75 days post transplant (82 days post accident).

Post mortem studies of the bone marrow showed $<10\%$ cellularity in total, with marked site-dependent variation. Macrophages were increased throughout and there was prominent erythrophagocytosis. Sparse hematopoietic foci were often of erythroblast predominance or immature myeloid cell predominance. There were fibroblasts with a swollen nucleus, particularly surrounding arterioli, where there was also swelling. A FISH analysis of bone marrow cells indicated 100% donor cells. There were no findings of extramedullary hematopoiesis. Other pathological findings were denudation of the epithelia of the skin and the gastrointestinal tract, sclerosis or fibrosis of subepithelial interstitium with bizzare-shaped fibroblasts, and dilatation of capillaries. Multi-organ failure was histologically evident. Post mortem results will be published in greater detail.

Discussion

Our subject received high-dose neutron-and γ-radiation. Exposure to high-dose neutron radiation is described in only six previous cases: Los Alamos, California and Rhode Island, USA, Constitu, Argentina and Sarov, Russia.[1,14] Because of the nature of neutron radiation, ie a non-charged particle beam with high linear energy transfer (LET), a sizable part of the energy is delivered to the tissues enriched with water and fat.[1,14] This contrasts with γ-radiation which distributes its energy more uniformly resulting in a different clinical presentation and therapy challenge.

Bone marrow transplants have been used to treat radiation victims since 1958.[5] Twenty-nine victims of five previous nuclear accidents received allo- ($n = 28$) or genetically identical twin ($n = 1$) transplants.[5–9] Nine survived >10 years post transplant. However, donor engraftment, although temporary, was confirmed in only two (Table 2). In seven others, engraftment of donor cells was indeterminate. Some data indicate that transplants shorten or reverse otherwise operationally irreversible bone marrow failure from radiation.[8,10,15] This scenario was shown in the Chernobyl radiation accident victims.[8] In our case, prolonged engraftment of donor cells did not result in long-term survival because of neutron- and γ-radiation-induced irreversible damage to the gastrointestinal tract and skin. Whether the bone marrow graft would have persisted is unknown. Also uncertain is the cause of the peri-terminal bone marrow failure. However, genetic analyses indicate that it was not graft rejection. Damage to the bone marrow microenvironment may also have contributed.

Three random chromosome breaks were found in three of 30 donor cells obtained from the sternal bone marrow. This is significantly above background levels where we found only two chromosome breaks in cells from normal subjects in over 150 000 metaphases analyzed. Recently, chromosome instability was reported in unexposed bone marrow cells transplanted simultaneously with the neutron-radiation exposed bone marrow cells into X-irradiated mice.[16] This 'bystander' effect has been explained by the theory that neutron radiation–exposed cells release reactive oxygen species that damage neighboring unexposed cells. This bystander effect may have occurred in our subject.[16–18]

In accidental high-dose total body radiation exposure, the calculus of whether to perform a transplant must include several factors: (1) likelihood of death from bone marrow failure without a transplant; (2) probability that transplant will decrease this risk; (3) likelihood of harm from a transplant; and (4) probability of death from non-bone marrow toxicity.[19] Our results indicate potential benefits, risks and complexities in analyzing the impact of interventions in radiation accident victims especially those exposed to high-dose neutron radiation. Because of this complexity and the unpredictable nature of radiation accidents, controlled evaluations of therapy interventions are impossible. Nevertheless, this case offers almost unlimited opportunities for studying radiation effects and the short-term, medium-term and long-term consequences. The subject is the first surviving so long after an essentially 'supra-lethal' radiation

Table 2 Nuclear accidents and hematopoietic stem cell transplant

	Year	*Transplants (n)*	*Dose (Gy)*	*Radiation source*	*Graft*	*Bone marrow recovery*	*Donor chimerism*	*Auto recovery*	*Survival*[a]	*Ref.*
Vinca (Yugoslavia)	1958	5	4.4	γ	FL + FS + BM[b]	Y	ND[c]	TETE[d]	32 d	5, 20
			4.3		BM	Y	Y(?), transient[e]	Y	⩾19 y	
			4.2		BM	Y	Y(?), transient	Y	⩾19 y	
			4.1		BM	Y	Y(?), transient	Y	⩾19 y	
			3.2		BM	Y	Y(?), transient	Y	⩾19 y	
An-Hui sheng (China)	1963	3	40	γ[f]	BM	TETE	NA[g]	NA	2 d	7
			8		BM	Y	ND	Y[h]	⩾14 y	
			6		BM	Y	ND	Y[h]	⩾14 y	
Pittsburgh (USA)	1967	1[i]	6	X-ray	BM	Y	NA	NA	⩾13 y	6
Chernobyl (USSR)	1986	13[j]	13.4	γ	BM	TETE	NA	NA	2 d	8
			12.1		BM	N	NA	NA	11 d	
			11.9		BM	N	NA	NA	11 d	
			10.2		BM	Y	Y[k]	Y[k]	79 d	
			9.6		BM	Y	Y[l]	TETE	11 d	
			9.2		BM	N	NA	NA	11 d	
			8.7		BM	Y	Y[k], transient	Y[k]	⩾15 y	
			8.3		BM	Y	Y[l]	Y[m]	11 d	
			6.6		BM	Y	NAn[n]	TETE	19 d	
			6.4		BM	Y	Y[k]	Y[k]	37 d	
			5.6		BM	Y	Y[k], transient	Y[k]	⩾15 y	
			5.2		BM	Y	Y[16]	Y[o]	77 d	
			4.4		BM	Y	Y[k]	Y[k]	27 d	
Soreq (Israel)	1990	1	10–18	γ[e]	BM	Y	Y[p]	TETE	32 d	9
Tokaimura (Japan)	1999	2	8.5–13/5.4	γ/neutron	Blood	Y	Y[k]	TETE	76 d	
			4.5–6.9/2.9		Cord blood	Y	Y[k], transient	Y[k]	201 d	

[a]Post transplant. [b]FL = fetal liver; FS = fetal spleen; BM = bone marrow. [c]Not documented. [d]Too early to evaluate. [e]Evaluated by red cell minor antigens, but not convincing. [f]Radiation source was [??0811]CO. [g]Not applicable. [h]No GVHD without prophylaxis and long-term survival indicate autologous recovery. [i]Genetically identical twin. [j]Fetal liver cells were transplanted to six other victims who died early post transplant because of severe thermal burn. [k]Evaluated by sex chromosomes. [l]Evaluated by appearance of normal chromosomes in sex-matched transplants. [m]Evaluated by the mixture of normal and abnormal chromosomes. [n]Not analyzed. [o]Evaluated by ABO marker in sex-matched transplant. [p]Evaluated by mismatched HLA marker.

exposure. Thus, it is important to document the results of therapy of this case for historical reference and future clinical decision-making.

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