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BIOLOGY CONTRIBUTION

COMPARISON OF AUTOLOGOUS CELL THERAPY AND GRANULOCYTE-COLONY STIMULATING FACTOR (G-CSF) INJECTION VS. G-CSF INJECTION ALONE FOR THE TREATMENT OF ACUTE RADIATION SYNDROME IN A NON-HUMAN PRIMATE MODEL

JEAN-MARC BERTHO, PH.D.,*† JOHANNA FRICK,*† MARIE PRAT,*† CHRISTELLE DEMARQUAY,*† NICOLAS DUDOIGNON,* FRANÇOIS TROMPIER,‡ NORBERT-CLAUDE GORIN, M.D., PH.D.,†§ DOMINIQUE THIERRY, PH.D.,*† AND PATRICK GOURMELON, M.D.*†

*Institut de Radioprotection et de Sûreté Nucléaire, Direction de la Radioprotection de l'Homme, Service de Radiobiologie et d'Epidémiologie, Laboratoire de Thérapie Cellulaire et de Radioprotection Accidentelle and ‡Service de Dosimétrie Externe, Fontenay-aux-Roses, France; †UPRES EA 1638, CHU Saint Antoine, Paris, France; §Service des Maladies du Sang, Hôpital Saint-Antoine, Paris, France

Purpose: To compare the efficacy of autologous cell therapy after irradiation combined with granulocyte-colony stimulating factor (G-CSF) injections with G-CSF treatment alone in a heterogeneous model of irradiation representative of an accidental situation.

Material and Methods: Non-human primates were irradiated at 8.7 Gy whole-body dose with the right arm shielded to receive 4.8 Gy. The first group of animals received G-CSF (lenograstim) injections starting 6 h after irradiation, and a second group received a combination of G-CSF (lenograstim) injections and autologous expanded hematopoietic cells. Animals were followed up for blood cell counts, circulating progenitors, and bone marrow cellularity.

Results: No significant differences were seen between the two treatment groups, whatever the parameter observed: time to leukocyte or platelet recovery and duration and severity of aplasia.

Conclusion: Our results indicated that identical recovery kinetic was observed when irradiated animals are treated with G-CSF independently of the reinjection of *ex vivo* expanded autologous hematopoietic cells. Thus G-CSF injections might be chosen as a first-line therapeutic strategy in the treatment of accidental acute radiation victims. © 2005 Elsevier Inc.

Acute radiation syndrome, Heterogeneous irradiation, Non-human primates, Cell therapy, G-CSF.

Although rare, radiation accidents induce a complex pathology named the acute radiation syndrome (ARS), which remains difficult to treat (1–5). Radiation victims develop a bone marrow (BM) aplasia in association with other pathologies such as a gastrointestinal damage and a cutaneous syndrome. The complexity of ARS may explain in part the relative limited efficacy of classic hematologic treatments such as cytokine injections or stem cell transplantation (3, 6, 7). A retrospective study of transplanted victims suggested a similar survival rate as compared with nontransplanted victims matched for the radiation dose (7). This was mainly because of the heterogeneity of accidental irradiation, which might preserve some hematopoietic cells able to reject the graft (4, 8). In fact, the transplantation of allogeneic cord blood stem cells in a victim of the Tokaimura radiation accident resulted in a transient engraftment followed by graft rejection and autologous recovery, despite a high radiation dose (4). Thus cytokine injections may be preferred to stem cell transplantation to promote endogenous hematopoietic recovery (9). The usefulness of granulocyte-colony stimulating factor (G-CSF) injections for the treatment of chemotherapy or radiotherapy-induced aplasia in the clinical setting has been proven for a long time (10–12). However, in radiation accident victims, the efficacy of cy-

Reprint requests to: Jean-Marc Bertho, PhD, Institut de Radioprotection et de Sûreté Nucléaire, Direction de la Radioprotection de l'Homme, Service de Radiobiologie et d'Epidémiologie, Laboratoire de Thérapie Cellulaire et de Radioprotection Accidentelle, B.P. no. 17, F-92262 Fontenay aux Roses cedex, France. Tel: (+33) 1-5835-9548; Fax: (+33) 1-5835-8467; E-mail: jean-marc.bertho@irsn.fr

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tokine injection remains difficult to assess, mainly because of the limited number of treated patients (6). Moreover, early-acting cytokines such as stem cell factor (SCF), Flt3 ligand (FL), and thrombopoietin, which showed a high efficacy in promoting hematopoietic recovery in animal models (13, 14) are not available for clinical use in humans (15).

Thus there is a need for new therapeutic protocols for the treatment of accidental radiation-induced BM aplasia. These protocols might take into account the two characteristics of accidental irradiations, i.e., the radiation heterogeneity and the complexity of ARS. One possible therapeutic strategy is autologous cell therapy that consists of the reinfusion of *ex vivo* expanded cells collected soon after irradiation in a less-irradiated BM territory. The high efficiency of *ex vivo* expanded cells to promote hematopoietic recovery was demonstrated in several studies both in animal models (16, 17) and in humans (18–20), with a complete abrogation of pancytopenia in some studies (20, 21). The application of such an autologous cell therapy protocol to the accidental irradiation situation is based on two main points. On the one hand, the existence of a residual hematopoiesis after heterogeneous irradiation, which was demonstrated in animal models (22, 23) and in humans (8, 24, 25), and on the other hand, on the ability of hematopoietic cells to expand *in vitro* after irradiation, which was also demonstrated in several studies (13, 26–28), although cell expansion was limited by increasing radiation dose. Overall, this indicated that an autologous cell therapy protocol is theoretically feasible after an accidental irradiation. Nevertheless, such strategy may be limited by both the extent of radiation heterogeneity and the radiation dose (22, 23). We have recently shown that an autologous cell therapy protocol was feasible after heterogeneous irradiation in a non-human primate model (29), and that the reinjection of expanded hematopoietic cells harvested after irradiation showed some ability to reduce the depth of radiation-induced aplasia. Another group also demonstrated in the mouse model that irradiated and cultured hematopoietic cells were able to rescue lethally irradiated animals (27). However, the therapeutic efficacy was limited not only by the development of a complex pathology involving several organs, but also by the low number of reinjected hematopoietic cells (27, 29). Thus in an attempt to improve hematopoietic recovery from reinjected cells, we tested a combination of two therapeutic approaches, namely autologous cell therapy and G-CSF (lenograstim) injections. This combination was compared with G-CSF injections alone in a model of heterogeneous irradiation of non-human primates. In this model, animals received a whole-body dose of 8.7 Gy with the right arm shielded to receive 4.7 Gy. The shielding to the right arm represents the partial protection of 4.5% of BM territory in this animal species (30). Such a heterogeneous irradiation is though to be representative of a heterogeneous accidental radiation overexposure (8, 24, 25).

METHODS AND MATERIALS

Animals

Male macaques (*Macaca fascicularis*), weighting mean 3.1 ± 0.3 kg, were housed in individual stainless steel cages with a room temperature of 23°C and 12 h/12 h night/day rhythm. Animals were provided with sterile water and biscuits *ad libitum* and peeled fruits. Irradiated animals were kept under laminar air flow. All experimental procedures were approved by the Animal Care Committee of the Institute of Radioprotection and Nuclear Safety and conformed to the French regulations for animal experimentation (Ministry of Agriculture Act No. 87-848, October 19, 1987; modified on May 29, 2001).

Animal irradiation and post-irradiation treatments

Animals were subjected to bacterial decontamination with oral administration of colistine (100 mg/kg/day Acti-coli B, Biové, Arques, France) and systemic antibiotic treatment (gentamicin 40 mg/kg/day and ampicillin 160 mg/kg/day, Bi-genta, Shering-Plough, Segré, France). The radiation was delivered with cobalt 60 sources (Commissariat à l'énergie atomique, Jouy en Josas, France), delivering a mean dose rate of 0.236 ± 0.005 Gy/min, which was the highest possible dose rate in this radiation device. Anesthetized animals received the irradiation dose in two successive parts. The first part was made with a lead shield covering the right arm up to the shoulder; the second part was done after removal of the lead shield, as previously described (29). Time for each part of the irradiation was calculated to obtain a mean body dose of 8.5 Gy, with a mean irradiation dose to the right arm of 4.5 Gy. These radiation doses were chosen to be close to the radiation dose used in a previous study (29). The shielding of the right arm allowed the protection of 4.5% of bone marrow territories in this animal species (30). A physical dosimetry by thermoluminescence dosimeters (31) was made to confirm radiation doses received by the animals (Table 1). Dexamethasone (5 μg/kg of body weight, Dexazone, UVA, Ivry sur Seine, France) was injected intravenously immediately after the end of irradiation to limit the initial acute inflammatory reaction. Antibiotic therapy was restarted 48 h after irradiation until neutrophil counts reached more than 0.5 ×

Table 1. Results of physical dosimetry

Anatomic location	Radiation dose	Anatomic location	Radiation dose	Anatomic location	Radiation dose
Right wrist	3.5 ± 0.4	Left wrist	9.1 ± 0.8	Iliac crest	9.1 ± 0.7
Right elbow	4.2 ± 0.3	Left elbow	9.7 ± 0.8	Ribs	8.7 ± 1.2
Right shoulder	6.5 ± 1.1	Left shoulder	9.1 ± 0.5	Thorax, front	8.4 ± 0.7
				Thorax, back	8.4 ± 0.7
Right arm, mean ± SD	4.8 ± 1.5	Left arm, mean ± SD	9.1 ± 0.8	Whole body, mean ± SD	8.7 ± 1.0

Each animal was fitted with 25 thermoluminescence dosimeters. The right arm was shielded during the first part of irradiation. Results are given as mean ± SD from individual values obtained for each animal and each anatomic location.