Human umbilical-cord-blood mononucleated cells enhance the survival of lethally irradiated mice: dosage and the window of time

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(Received 31 January 2013; revised 5 April 2013; accepted 12 April 2013)

The purpose of this study was to evaluate the window of time and dose of human umbilical-cord-blood (HUCB) mononucleated cells necessary for successful treatment of radiation injury in mice. Female A/J mice (27–30 weeks old) were exposed to an absorbed dose of 9–10 Gy of 137Cs γ-rays delivered acutely to the whole body. They were treated either with 1 × 10^8 or 2 × 10^8 HUCB mononucleated cells at 24–52 h after the irradiation. The antibiotic Levaquin was applied 4 h postirradiation. The increased dose of cord-blood cells resulted in enhanced survival. The enhancement of survival in animals that received 2 × 10^8 HUCB mononucleated cells relative to irradiated but untreated animals was highly significant (P < 0.01). Compared with earlier studies, the increased dose of HUCB mononucleated cells, coupled with early use of an antibiotic, extended the window of time for effective treatment of severe radiation injury from 4 to 24–52 h after exposure.

Keywords: acute lethal total-body irradiation; human umbilical-cord blood; antibiotics; radiation mass casualties

INTRODUCTION

The growing threat of terrorist activities employing radiological weapons has become a national security priority. Acute radiation syndrome (ARS) is a likely outcome that could affect a large cohort of individuals after exposure to ionizing radiation (IR). Thus, the development of countermeasures that could be used to treat mass casualties from the harmful effects of IR is of eminent importance. As a consequence, numerous national and international agencies are actively working on developing suitable plans for clinical management of radiologic victims. A major focus is on identifying mitigators of radiation injury that could be effectively used during a realistic period of time following the exposure.

In the event of a nuclear explosion, symptoms of the ARS (nausea, vomiting and diarrhea), due to damage to the immune system, the gastrointestinal tract and other organs, would likely be manifested in a large number of victims. ARS can occur within hours after whole body irradiation with relatively moderate acute absorbed doses. To repair damage to the immune system, human-leukocyte-antigen-(HLA-) matched bone-marrow transplantation would be ideal. However, it would not be technically possible in the case of mass casualties when hundreds, if not thousands, of bone-marrow transplants would need to be performed. Medical facilities would be overwhelmed in the first hours and days after the explosion; doctors might not be able to perform the necessary laboratory tests to evaluate the damage to the bone marrow in each patient and find suitable HLA-matched donors.

A readily available source of stem cells is human umbilical-cord blood (HUCB). Fresh or frozen units of HUCB, matched by blood type only, can be a valuable alternative to bone-marrow transplantation for restoring an immune system damaged by irradiation [1]. In fact, in 2005, the establishment of National Cord-Blood Depositories was legislated in the USA for the purpose of restoring immune function following a nuclear explosion from a terrorist attack or an accident. In 2010, the law was amended to ensure the storage of a minimum of 150 000 units [2, 3]. Victims of lethal irradiation...
who are treated with HUCB transfusions, coupled with antibiotics, may benefit both from the transplantation effect and also an endogenous effect, stimulating repair of systemic damage. This therapy may also contribute to recovery from trauma and burn injuries that radiation victims may sustain [4].

HUCB has been given to patients since 1914 [5] and the safety of the therapy is well documented [6]. In 1972, we reported the first recorded HUCB transplantation with minimal immune suppression. A total of 139 ABO (blood type) partially compatible fetal-cord-blood transfusions were given to 15 patients with terminal illness, primarily with hematological malignancies, without any adverse reaction [7, 8]. The transplantation procedures were performed after obtaining informed signed consent from each patient (there were no institutional review boards at the time). In subsequent experiments with mice, we showed that administration of HUCB and antibiotics 4 h after lethal irradiation enhanced survival and restored damage to the bone marrow and gastrointestinal tract [9–11]. These results, together with analysis of pathologies of Hiroshima and Nagasaki victims [12, 13], suggest that many human lives could be saved with antibiotics and HUCB therapy. However, the realistic scenario is that treatment of radiological mass casualties will require a prolonged period of time, at least 24–72 h, to deliver medical help to the victims. Hence, in this study we explored whether an increased dose of mononucleated HUCB cells coupled with antibiotic therapy could extend the window of time available for successful treatment of injury after acute lethal total body irradiation in mice.

### MATERIALS AND METHODS

The study protocol was reviewed and approved by the Institutional Animal Care and Use Committee of the University of Medicine and Dentistry of New Jersey – New Jersey Medical School. Female A/J mice (27–30 weeks old) described as moderately radiosensitive (LD50/30 ~6.75 Gy) [14] were divided into six experimental groups: (i) sham-irradiated control: 6 mice; (ii) irradiation alone: 9 mice; (iii) irradiated and treated with 1 × 10^8 HUCB mononuclear cells: 11 mice; (iv) irradiated and treated with the antibiotic Levaquin: 12 mice; (v) irradiated and treated with antibiotic and 1 × 10^6 HUCB mononuclear cells: 12 mice; (vi) irradiated and treated with antibiotic and 2 × 10^6 HUCB mononuclear cells: 16 mice.

Animals were exposed to an absorbed dose of 9–10 Gy (1 Gy/min) γ rays delivered to the whole body from a 137Cs source in a ventilated irradiator (JL Shepherd, Mark I, San Fernando, CA) located within the vivarium. The mice were placed in a multichamber device (a single mouse per chamber) on a rotating platform to ensure uniform exposure. Control mice were sham-treated. Mice in each group were equipped with individual dosimeters to verify the delivered dose (Mirion Technologies, Irvine, CA). The average absorbed doses were not statistically different between the experimental groups (Table 1).

The antibiotic Levaquin (Janssen Pharmaceuticals, Inc., Raritan, NJ) was administered in drinking water (0.1 mg/ml) 4 h following the irradiation. Multiple cord-blood units of the same blood type were combined for injection procedures. Frozen red-cell-depleted HUCB units (Celgene Cellular Therapeutics, Cedar Knolls, NJ) were processed as previously described [9]. To deliver 1 × 10^6 HUCB mononuclear cells, the animals were injected retro-orbitally with 0.5 × 10^8 cells per injection at 24 and at 30 h following the irradiation. To deliver 2 × 10^6 cells, injections of 0.5 × 10^8 cells were given at 24, 30, 48 and 52 h after the irradiation (see schematic in Fig. 1). Multiple injections were necessary to prevent volume overload in animals.

Mice were weighed prior to irradiation and then at weekly intervals. The animal facility at the New Jersey Medical School is a specific pathogen-free barrier facility. Animals were placed in sterile cages immediately after completion of HUCB injections and every time after weighing to simulate reverse isolation. The food (sterile Purina rodent chow) and sterile drinking water were given ad libitum. To assess survival, the animals were monitored for 50 days after the irradiation until euthanized.

The method of Kaplan–Meier was used to estimate the distribution of the percentage of survival over time. Student’s t-test was used to assess statistical differences in absorbed dose of radiation between the experimental groups.

### Table 1. Absorbed doses of 137Cs γ rays delivered acutely (1 Gy/min) to the whole body of A/J female mice (27–30 weeks old)

| Group | Animals per group (n) | Average dose Gy | SD (Gy) |
|-------|-----------------------|------------------|---------|
| IR    | 9                     | 9.0              | 1.0     |
| IR + HUCB (1 × 10^8 MNC) | 11            | 9.3              | 1.1     |
| IR + Levaquin | 12            | 9.6              | 0.9     |
| IR + Levaquin + HUCB (1 × 10^6 MNC) | 12           | 8.9              | 1.3     |
| IR + Levaquin + HUCB (2 × 10^6 MNC) | 16            | 9.0              | 1.1     |

IR = irradiated and untreated animals, IR + HUCB (1 × 10^8 MNC) = irradiated and treated with 1 × 10^8 HUCB mononuclear cells, IR + Levaquin = irradiated and treated with antibiotic, IR + Levaquin + HUCB (1 × 10^6 MNC) = irradiated and treated with antibiotic and 1 × 10^6 HUCB mononucleated cells, IR + Levaquin + HUCB (2 × 10^6 MNC) = irradiated and treated with antibiotic and 2 × 10^6 HUCB mononucleated cells. (Student’s t-test was applied to confirm that there was no significant difference in the γ-ray-absorbed dose between the experimental groups.)
Fisher’s exact test (two-tailed) was used to estimate the statistical significance in survival differences between experimental groups. A $P$ value of less than 0.05 was considered statistically significant.

**RESULTS**

Increasing the dosage of cord-blood cells extends the window of time for treatment of lethal radiation injuries

$\text{A/J mice exposed to an absorbed dose of } 9 \pm 1 \text{ Gy from } ^{137}\text{Cs } \gamma \text{ rays but not treated with antibiotics or cord blood generally died within 24–42 days. Unexpectedly, a small fraction of animals survived for } > 50 \text{ days (two out of nine), which may be attributed to the older age of the mice compared with those in our earlier studies (27–30 weeks versus 9 weeks) [9]. Compared with the radiation-only group, a significantly increased survival was observed in mice exposed to } 9 \pm 1.1 \text{ Gy and treated with Levaquin and } 2 \times 10^8 \text{ HUCB mononucleated cells administered within 24–52 h after irradiation (} P < 0.01) \text{. More than 93% of irradiated animals treated with } 2 \times 10^8 \text{ HUCB cells and Levaquin survived till euthanized at 50 days (Fig. 2). On the other hand, more than 66% of animals that received the smaller dose of HUCB cells (i.e. } 1 \times 10^8 \text{) and Levaquin survived for the 50 days, although the result did not reach statistical significance when compared with the radiation-only group (} P = 0.08) \text{. Notably, 58% of mice that received only Levaquin following irradiation survived for the 50 days; in contrast, 36% of irradiated animals treated with cord blood alone survived the 50 days (Fig. 2). Although, the differences in survival were non-significant, the latter observation highlights the important role of early administration of antibiotics in survival. This effect likely occurs through prevention of infection, recovery of the immune system and restoration of body functions. Nevertheless, greater survival was achieved when antibiotic administration was combined with HUCB (} P = 0.06 \text{, irradiated groups treated with Levaquin only vs Levaquin and } 2 \times 10^8 \text{ HUCB cells).}\text{ After irradiation all the animals lost weight; those that survived for the 50 days regained weight, returning to close to the initial weight as measured prior to irradiation. No significant differences were observed between the groups of treated animals in terms of weight loss or recovery.}\text{**DISCUSSION**

HLA-matched bone-marrow transplantation following accidental acute high-dose total-body exposure to neutrons and $\gamma$-rays has been used since 1958, albeit for a small number of victims [15]. In the event of radiologic/nuclear mass casualties, HUCB transplantation offers advantages over HLA-matched bone-marrow transplantation, since cord blood is more readily available (stored and cataloged in blood banks). Moreover, HUCB can be quickly identified and transplanted, matched by blood type only. Importantly, lower-than-anticipated risk of
severe acute graft-versus-host disease makes cord-blood transplantations a valuable source of hematopoietic stem cells that can repopulate damaged bone marrow [16]. The HUCB cells allow the host to recover its own hematopoietic system and restore immunocompetence, possibly through secretion of growth-promoting factors [17, 18]. However, successful engraftment of HUCB in mice may not be achieved. There has only been one case where cord-blood transplantation was used after accidental exposure to IR. A worker at a nuclear fuel processing plant in Tokaimura (Ibaraki, Japan) received a supralethal dose of neutrons and $\gamma$-rays. On the ninth day after the incident, he was treated with HLA-DRB1 (major histocompatibility complex, Class II, DR beta 1) one unit of locus-mismatched unrelated umbilical cord blood, and lived for 210 days. The victim died from complications, namely skin burns and infections. It is worth noting that the patient’s immune system was showing signs of recovery, even though he received only one unit of cord blood [19].

Our current study has shown that the increased number of HUCB mononucleated cells obtained from multiple units of human cord blood extends the window of time for successful treatment of radiation-induced injury caused by 9–10 Gy of $\gamma$ rays delivered acutely to the whole body of middle-aged female A/J mice. The administration of $2 \times 10^8$ HUCB mononucleated cells within 24–52 h following the irradiation, coupled with the antibiotic Levaquin, significantly enhanced the probability of survival compared with irradiated and untreated animals. The early use of an antibiotic likely protected animals from complications of infections, thus enabling cord-blood cells to be effective in enhancing survival and restoration of normal body functions. Over 90% of mice treated with Levaquin within 4 h after exposure, and HUCB cells within 24–52 h, lived for over 50 days until euthanized. These findings extend our previous results [9] indicating the radioprotective effects of antibiotic and cord-blood therapy when administered early (4 h) after exposure.
Here we show that increasing the number of HUCB cells enhances the window of opportunity for successful treatment of radiation injury, which would be critical for executing medical plans on a mass casualty scale. The findings of these studies may also be useful in determining treatment of life-threatening complications from radio- and chemotherapy in cancer patients.

ACKNOWLEDGEMENTS

We thank Dr George Yap (New Jersey Medical School) for helping with cord-blood injections and Dr Manoochehr Khorshidi (Celgene Cellular Therapeutics) for providing cord-blood units for our experiments. The authors alone are responsible for the content and writing of the paper.

FUNDING

This work was supported by the Abraham S. Ende Research Foundation and by Grant CA049062 from the National Institutes of Health.

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