

New emerging concepts in the medical management of local radiation injury

Emerging concept of local radiation treatment

Marc Benderitter^a, Jean Jacques Lataillade^b, Eric Bey^c, Marie Prat^b and Patrick Gourmelon^a

^a *Institut de Radioprotection et de Sûreté Nucléaire, Laboratoire de radiopathologie et de thérapie cellulaire, BP 17, 92262 Fontenay-aux-Roses, France.*

^b *Centre de transfusion sanguine des armées, Département de recherche et de thérapie cellulaire, 92141 Clamart, France.*

^c *Service de Chirurgie plastique, Hôpital d'Instruction des Armées Percy, 92141 Clamart, France.*

Abstract

Treatment of severe radiation burns remains a difficult medical challenge. The response of the skin to ionizing radiation results in a range of clinical manifestations. The most severe manifestations are highly invalidating. Although several therapeutic strategies (excision, skin grafting, skin or muscle flaps) have been used with some success, none have proven entirely satisfying. The concept that stem cell injections could be used for reducing normal tissue injury has been discussed for a number of years. Mesenchymal Stem Cells (MSC) therapy may be a promising therapeutic approach to improve radiation-induced skin and muscle damages. Pre-clinical and clinical benefit of MSC injection for ulcerated skin and muscle restoration after high dose radiation exposure has been successfully demonstrated. Three first patients suffering from severe radiological syndrome were successfully treated in France based on combined autologous human grade MSC injection to plastic surgery. Stem cell therapy must be now improved to the point that hospitals can put safe, efficient, reliable and inexpensive clinical protocols into practice.

KEYWORDS: *Stem cell therapy, Mesenchymal stem cell, radiological burn*

1. Scientific and clinical background

The medical management of severe radiation burns after accidental overexposure to ionizing radiation is still a major therapeutic challenge [1] unresolved with the classical therapeutic approach derived from the management of thermal or electrical burns.

There are marked differences between radiation and thermal burns in terms of patho-physiological mechanisms, clinical aspects and evolution. The main feature of severe radiation burns is the occurrence of unpredictable successive inflammatory waves leading to the extension, in surface and in depth, of the necrotic process. After an initial period marked by a clinical picture limited to a rash and itching, subsequent ulceration and necrosis develop, which may extend to the deep dermal and underlying muscle structures. Moreover, inflammatory waves are associated with uncontrollable pain highly resistant to morphinics. The patho-physiological process, which involves a cascade of inflammatory mediators and a continuous activation of target cells (endothelial cells and fibroblasts), is not totally elucidated [2-6].

The surgical management of severe necrotic radiation burns is theoretically easy to perform. The conventional main strategy is the excision of the necrotic tissues followed by a rotation flap or a good quality skin autograft. In practice, the planning of such a surgical approach often encounters insurmountable technical difficulties due to the occurrence of successive and unpredictable inflammatory waves associated with a progressive extension of the necrotic process. Then, the evolution of the radiation lesion often becomes uncontrolled and the final option is a last surgical act leading to a very high morbidity and disability. Thus, in two highly irradiated Peruvian and Georgian victims, previously treated in 1999 and 2002, with the classical surgical approach combining excision and skin graft, it was not possible, in the Peruvian case, even after amputation of the irradiated leg, to manage the huge extension, at the perineal level, of the radionecrotic process. Concerning the Georgian case, the conventional treatment was a failure since four successive excisions followed by skin autografts were always inefficient 440 days post-exposure and only an autonomous, vascularized tissue (omentum flap) covered by skin allograft allowed healing at 500 days PI [7-8].

Thus, to date, the best therapeutic approach for severe radiation burns remains unknown.

2. New emerging concept

Irradiation kills normal cells directly or indirectly and the basic issue is to replace them. Replenishment of the depleted stem cell compartment and/or stem cell plasticity should allow better regeneration of irradiated tissues. Adult stem cell therapy was postulated to favour also the radiation burn healing process through the secretion of trophic factors (growth factors, cytokines) that may counteract the local inflammatory wave processes and favour angiogenesis. Stem cell therapy using bone marrow mesenchymal stem cells (MSC) may be a promising therapeutic approach to improve radiation-induced normal tissue damage.

The first challenge in MSC transplantation is that the cultured cells retain their quality and their differentiation potential during the expansion process. In order to treat tissue injury using cell therapy, the number of cells required can be very high. Stem cells are a small percentage of the total cellularity, so their pool has to be expanded *ex vivo* and injected taking into account the need for immature cells – stem cells and progenitors – or differentiated cells. To be of therapeutic use, the produced cells must retain normal function, differentiation pattern and regulation during culture.

Mesenchymal stem cells (MSC) have been described in the bone marrow as multipotent progenitor cells that differentiate into endothelial cells, epithelial cells, stromal cells but also osteocytes, chondrocytes and adipocytes. Their ability to differentiate according to multiple lineage characteristics is preserved along the expansion process. It has been demonstrated that MSCs can be easily recovered from bone marrow or adipose tissue and enriched through their property of adhering to tissue culture surfaces. Several groups have recently expanded MSCs up to a million fold *in vitro* for hematologically and orthopedically relevant applications.

Moreover, MSCs are able to migrate towards injured tissular lesions where they deliver a high number of growth factors that are required for immunoregulation and repair processes [9, 13]. Their positive effect in promoting the healing of radiation burn lesions in a preclinical immunodeficient non obese diabetic/severe combined immunodeficient (NOD/SCID) mouse model was demonstrated [10-13]. In this model, the intravenous administration of human MSCs strongly improved the healing of burn lesions induced by a 30 Gy irradiation [13]. The presence of hMSC in the mouse skin 21 days after transplant suggest that incorporation of transplanted cells in skin structure can only be seen 3 weeks after wounding. Once implanted in the injured area, bone marrow cells could promote the migration, proliferation and differentiation of epidermal cells. The presence of hMSC was evidenced in the irradiated epidermis. These results are the first evidence, using human cells, of the possible use of human MSC for the treatment of the acute cutaneous radiation syndrome [13].

The mechanisms leading to the observed positive effects of hMSC therapy on skin repair remain to be studied. It has been reported that bone marrow cells could become perifollicular cells, blood vessel cells or perisebaceous gland cells during the healing process [14]. Bone marrow cells could differentiate into myofibroblasts and play an important role in the formation of granulation tissue during the wound healing process when combined with an occlusive dressing. Injected bone marrow MSC could give rise to functional skin cells at a high frequency and regenerate skin tissue. Furthermore, in a skin defect model, MSC associated with Fibroblast Growth Factor (FGF) could accelerate cutaneous wound healing as MSC differentiate into the epithelium [15]. The production and secretion of beta-TGF by hMSC could be involved, suggesting a possible effect of hMSC on the skin lesion through paracrine mediator release. Indeed, it has been reported that beta-TGF may induce the growth of stem cells at the level of the skin and may stimulate the repair process by enhancing extra-cellular matrix synthesis [16-18].

The successful transplant of stem cells and subsequent reduction in radiation-induced complications may open the road to completely new strategies in cutaneous radiation syndrome therapy. All together, these results are of clinical significance, as a drastic reduction of skin necrosis may be a major advance in the treatment of acute cutaneous radiation reactions. This work supports the use of hMSC infusion to repair skin injuries in patients after accidental irradiation.

3. Medical breakthrough in the treatment of radiological burn : 2 case reports

Clinical case report 1: The accident of Conception (Chili-2005) [20-21]

A 27-year-old Chilean man was overexposed, on 15 December 2005, to a gammagraphy radioactive source (^{192}Ir , 3.3 TBq). He picked up the source with his left hand and put it in the back left pocket of his trousers. Following a multifocal localized irradiation, he rapidly exhibited severe radiation burns located to the hand and the buttock. The early occurrence of skin lesions (ringed permanent erythema with a central atonic area) at the buttock level within the first days after irradiation strongly suggested a very high-dose exposure. The buttock skin lesion evolved into moist epidermitis (4–5 cm in diameter), then quickly worsened and progressed to ulceration. These radiation skin lesions were accompanied by classical intense pain, which was only partially

alleviated by morphine. The early development of the buttock lesion without any latency phase, its fast evolution toward ulceration and the uncontrolled pain were characteristic of a very severe radiation burn with poor prognosis.

The strategy adopted to reconstruct the accidental dose distribution delivered to the patient was based on numerical simulations. The numerical dosimetric reconstruction of the radiation accident requires simulating the source, its environment and the patient body using a numerical anthropomorphic phantom. The doses absorbed by the tissues were then calculated using a computer code. The dose absorbed at the skin lesion center was very high (almost 2000 Gy), but dropped rapidly due to the combined effect of distance and tissue attenuation. Based on the dose reconstruction mapping, a wide resection in apparently healthy tissues was performed on day 21 PI. All tissues exposed to a dose over 20 Gy that were situated between the center of the lesion and the 20 Gy isodose were excised according to hemisphere of 10 cm in diameter and then covered with a cryopreserved allograft.

A new therapeutic strategy combining this classical surgery procedure (excision plus skin autograft) and a local MSC therapy was designed. For MSC production, an autologous bone marrow collection was performed, allowing a two-step MSC expansion producing 182×10^6 cells at the first passage (P1) and 227×10^6 cells at the second passage (P2). Expanded cells exhibited antigenic characteristics of MSCs: they did not express CD45, but expressed CD90, CD105 and CD73 antigens. MSC purity was up to 97% and their clonogenic efficiency obtained at P1 was 225. No telomerase activity was found in the expanded MSCs. The pluripotentiality of expanded MSC was controlled by *in vitro* osteogenic, adipogenic and chondrogenic assays. Local administrations of 168×10^6 MSC on day 90 PI and 226×10^6 on day 99 PI were performed in circle around the lesion at the cutaneous and muscular levels, and in the wound bed of the lesion under the skin graft. The lesion was further dressed with an artificial derma (Integra®). Following this combined therapy, the healing of the lesion proceeded smoothly. There was no side effect. Optimal healing persists 2 years after the procedure.

Clinical case report 2: The accident of Dakar (Senegal-2006) [19]

A second experience of therapeutic management of a radiation accident victim combining stem cell therapy using autologous mesenchymal stem cells and surgery is reported. On 3 June 2006, in Dakar, during a gammagraphy operation, whilst inserting the source in the storage container, the end of the remote control broke off for unknown reasons. Due to this technical failure, the ^{192}Ir radioactive source was lodged unexpectedly in the source ejection duct instead of being returned to its shielded storage container, without this malfunction being detected. The remote control and the ejection duct (holding the source) were stored temporarily under the stairs in an entrance hall. Four patients were irradiated and sent to the Percy hospital in France. The most severe irradiated patient presented a very severe arm radiation burn which was treated by several surgical times/ iterative excision, skin graft, latissimus muscle dorsi flap and forearm radial flap. Local autologous MSC were administered as an adjuvant to improve the surgical approach. The clinical evolution, radiation pain and healing progression was favourable and no recurrence of radiation inflammatory waves was observed during the eight month patient's follow-up suggesting that MSC act as 'cell drug' in modulating radiation inflammatory processes.

4. Conclusion

In conclusion, these data and first clinical application open new prospects in the medical management of severe radiation burns. If confirmed in further radiation accidents, it would bring a major therapeutic improvement. Stem cell therapy must be improved to the point that hospitals can put safe, efficient and reliable and inexpensive clinical protocols into practice. The Institute of Radioprotection and Nuclear Safety (IRSN) develops procedures that should achieved tissue repair in the long term to the benefice of a great number of patients with skin complications after high dose radiation exposure. Furthermore, this novel multidisciplinary therapeutic approach using physical techniques, surgical procedures and cellular therapy with adult stem cells may open new prospects in the field of radiotherapy complications.

REFERENCES

- [1] Porock D, S Nokoletti and L Kristjanson. (1999). Management of radiation skin reactions: literature review and clinical application. *Plast Surg Nurs* 19: 185-192.
- [2] Milliat F, François A, Isoir M, Deutsch E, Tamarat R, Tarlet G, Atfi A, Validire P, Bourhis J, Sabourin JC, Benderitter M. Influence of endothelial cells on vascular smooth muscle cells phenotype after irradiation: implication in radiation-induced vascular damages. *Am J Pathol.* 2006; 169(4):1484-95.
- [3] Müller K, Köhn FM, Port M, Abend M, Molls M, Ring J, Meineke V. Intercellular adhesion molecule-1: a consistent inflammatory marker of the cutaneous radiation reaction both in vitro and in vivo. *Br J Dermatol.* 2006 155(4):670-9.
- [4] Benderitter M, Isoir M, Buard V, Durand V, Linard C, Vozenin-Brottons MC, Steffanazi J, Carsin H, Gourmelon P. Collapse of skin antioxidant status during the subacute period of cutaneous radiation syndrome: a case report. *Radiat Res.* 2007;167(1):43-50.
- [5] Flanders KC, Ho BM, Arany PR, Stuelten C, Mamura M, Paterniti MO, Sowers A, Mitchell JB, Roberts AB. Absence of Smad3 induces neutrophil migration after cutaneous irradiation: possible contribution to subsequent radioprotection. *Am J Pathol.* 2008; 173(1):68-76.
- [6] Holler V, Buard V, Gaugler MH, Guipaud O; Baudelin C, Sache A, del R Perez M, Squiban C, Tamarat R, Milliat F and Benderitter M, Pravastatin limits the radiation-induced vascular dysfunction in the skin. *JID* 2008 (in press).
- [7] INTERNATIONAL ATOMIC ENERGY AGENCY, The radiological accident in Lilo. IAEA Vienna (2000)
- [8] INTERNATIONAL ATOMIC ENERGY AGENCY, The radiological accident in Yanango. IAEA Vienna (2000)
- [9] Chapel A, J M Bertho, M Bensidhoum, L Fouillard, R G. Young, J Frick, C Demarquay, F Cuvelier, E Mathieu, F Trompier, N Dudoignon, C Germain, C Mazurier, J Aigueperse, J Borneman, N Cl Gorin, P Gourmelon and D Thierry. (2003). Mesenchymal stem cells home to injured tissues when co-infused with haematopoietic cells to treat a radiation-induced multi-organ failure syndrome. *J Gene Med* 5(12): 1028-1038.
- [10] Dantzer D, P Ferguson, RP Hill, A Keating, RA Kandel, JS Wunder, B O'Sullivan, J Sandhu, J Waddell and RS Bell. (2003). Effect of radiation and cell implantation on wound healing in a rat model. *J Surg Oncol* 83(3): 185-190.
- [11] Satoh H, K Kishi, T Tanaka, Y Kubota, T Nakajima, Y Akasaka and T Ishii. (2004). Transplanted mesenchymal stem cells are effective for skin regeneration in acute cutaneous wounds. *Cell Transplant* 13(4): 405-412.
- [12] François S, M Mouiseddine, N Mathieu, A Semont, P Monti, N Dudoignon, A Saché, A Boutarfá, D Thierry, P Gourmelon, A Chapel. Human mesenchymal stem cells favour healing of the cutaneous radiation syndrome in Non Obese Diabetes/Severe Combined a xenogenic transplant model. Immunodeficiency mouse model. *Annals of Hematology* 2007, 86: 1-8
- [13] François S, M Bensidhoum, M Mouiseddine, C Mazurier, B Allenet, A Semont, J Frick, A Saché, S Bouchet, D Thierry, P Gourmelon, N Gorin, Chapel A. Local irradiation induces not only homing of human Mesenchymal Stem Cells (hMSC) at exposed sites but promotes their widespread engraftment to multiple organs: A study of their quantitative distribution following irradiation damages. *Stem Cells* 2006, 24:1020-9.
- [14] Badavias E, M Abedi, J Butmarc, V Falanga and P Quesenberry. (2003). Participation of bone marrow derived cells in cutaneous wound healing. *J Cell Physiol* 196:245-250.
- [15] Yamaguchi Y, T Kubo, T Murakami, M Takahashi, Y Hakamata, E Kobayashi, S Yoshida, K Hosokawa, K Yoshikawa and S Itami. (2005). Bone marrow cells differentiate into wound myofibroblasts and accelerate the healing of wounds with exposed bones when combined with an occlusive dressing. *Br J Dermatol* 152:616-622.
- [16] Deng W, Q Han, L Lioa, C Li, W Ge, Z Zhao, S You, H Deng, F Murad and R Zhao. (2005). Engrafted bone marrow-derived Flk-1+ mesenchymal stem cells regenerate skin tissue. *Tissue Engineering* 11(1/2): 110-119.
- [17] Nakagawa H, S Akita, M Fukui, T Fujii, K Akino. (2005). Human mesenchymal stem cells successfully improve skin-substitute wound healing. *Brit J Dermatol* 153: 29-36.
- [18] Satoh H, Kishi K, Tanaka T, Kubota Y, Nakajima T, Akasaka Y, Ishii T (2004) Transplanted mesenchymal stem cells are effective for skin regeneration in acute cutaneous wounds. *Cell Transplant* 13(4):405-412
- [19] Bey E, Duhamel P, Lataillade JJ, de Revel T, Carsin H, Gourmelon P (2007). Treatment of radiation burns with surgery and cell therapy. A report of two cases. *Bull Acad Natl Med.*; 191(6):971-8.
- [20] Lataillade JJ, Doucet C, Bey E, Carsin H, Huet C, Clairand I, Bottollier-Depois, JF, Chapel A, Ernou I, Gourven M, Boutin L, Hayden A, Carcamo C, Buglova E, Joussemet M, de Revel T, Gourmelon P (2007). New approach to radiation burn treatment by dosimetry-guided surgery combined with autologous mesenchymal stem cell therapy. *Regen Med.*;2(5):785-94.
- [21] INTERNATIONAL ATOMIC ENERGY AGENCY, The radiological accident in Coception. IAEA Vienna (in press)