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A biomaterial-assisted mesenchymal stromal cell therapy alleviates colonic radiation-induced damage

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ABSTRACT

Healthy tissues surrounding abdomino-pelvic tumours can be impaired by radiotherapy, leading to chronic gastrointestinal complications with substantial mortality. Adipose-derived Mesenchymal Stromal Cells (Ad-MSCs) represent a promising strategy to reduce intestinal lesions. However, systemic administration of Ad-MSCs results in low cell engraftment within the injured tissue. Biomaterials, able to encapsulate and withstand Ad-MSCs, can overcome these limitations. A silanized hydroxypropylmethyl cellulose (Si-HPMC) hydrogel has been designed and characterized for injectable cell delivery using the operative catheter of a colonoscope. We demonstrated that hydrogel loaded-Ad-MSCs were viable, able to secrete trophic factors and responsive to the inflammatory environment. In a rat model of radiation-induced severe colonic damage, Ad-MSC + Si-HPMC improve colonic epithelial structure and hyperpermeability compared with Ad-MSCs injected intravenously or locally. This therapeutic benefit is associated with greater engraftment of Si-HPMC-embedded Ad-MSCs in the irradiated colonic mucosa. Moreover, macrophage infiltration near the injection site was less pronounced when Ad-MSCs were embedded in the hydrogel. Si-HPMC induces modulation of chemoattractant secretion by Ad-MSCs that could contribute to the decrease in macrophage infiltrate. Si-HPMC is suitable for cell delivery by colonoscopy and induces protection of Ad-MSCs in the tissue potentiating their therapeutic effect and could be proposed to patients suffering from colon diseases.

1. Introduction

Radiotherapy plays a crucial in the management of malignant pelvic diseases. However, the major limitation of this treatment is its toxicity on surrounding healthy tissues such as the small intestine, colon and rectum. Due to the high proliferative capacity of epithelial stem cells located at the base of the crypts, the gut is a very radiosensitive organ. Irradiation produces free radicals that, through direct and indirect effects, induce crypt cell apoptosis leading to mucosal lesions with a loss of epithelial barrier function. The intestinal epithelial barrier is maintained by intracellular junctional complexes, such as tight junctions (TJs), adherent junctions and desmosomes. Irradiation increases mucosal permeability, inducing nutrient and fluid loss as well as gut pathogen infiltration, exacerbating mucosal inflammation [1]. This inflammatory state, combined with the deficiency of epithelial stem cells and local ischemia, leads to a defective healing process which can result in tissue loss (ulceration) or pathological healing (fibrosis, fistula) [2].

Patients (10–20%) undergoing radiotherapy for abdominal cancers develop intestinal complications several years after the end

Abbreviations: Ad-MSCs, Adipose-derived Mesenchymal Stromal Cells; Si-HPMC, Silanized HydroxyPropylMethyl Cellulose.

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of their treatment. These chronic complications adversely affect quality of life and, in some cases, can be life-threatening. Management of patients suffering from radiotherapy-related gastrointestinal disorders is limited to symptomatic treatments. No curative treatments are currently available. Therefore, symptoms reoccur once treatment is stopped and new features may arise depending upon the course of the disease. The lack of curative treatment and the potential severity of the gastrointestinal disorders highlight the importance of finding a novel and effective treatment. Stem cell-based regenerative medicine using mesenchymal stromal cells (MSCs) is considered a promising approach, as suggested by encouraging results from various clinical trials [1].

Convincing experimental results from different animal models [3–5] and in clinical cases treated on a compassionate basis [6,7] have shown that intravenous (IV) injection of MSCs reduces severe colorectal lesions caused by ionizing irradiation. Experiments performed on animal models have shown that IV-MSC treatment promotes epithelial regeneration leading to intestinal structure improvement and limiting the fibrosis process [3–5]. The beneficial effects of MSC injection have been primarily related to their secretion of a wide variety of bioactive molecules [8]. MSCs secrete angiogenic, pro-regenerative, anti-apoptotic and immunosuppressive factors that may contribute to intestinal ulcer wound healing. Moreover, pre-clinical data on mini-pigs has demonstrated that repeated intravenous MSC injections are necessary to improve radio-induced colorectal ulcer healing [9]. These data suggest that MSC-induced therapeutic benefit requires a large number of injected cells. Despite a large amount of MSCs being systemically injected, very low cell engraftment in the irradiated intestine has been reported [3,4]. Indeed, studies have demonstrated that systemically injected MSCs were trapped in the lung after infusion [10]. Although a study from Prockop group has demonstrated that the anti-inflammatory effects of MSCs depend upon their trapping in the lung [11], some studies have demonstrated a pulmonary embolus following intravenous injection of MSCs [12–14]. It is known that MSCs are short-lived when infused intravenously. Indeed, they die within 24 h and are cleared from the body [15]. Thus, local injection of cells has been tested according to the accessibility of the tissue [16]. This injection method allows fewer cells to be injected, increases the rate of MSC engraftment in the area requiring repair and interestingly, for clinical application, reduces the cost of the treatment. Moreover, local injection reduces the spread of stem cells throughout the body, although no side effects have been shown after either local or intravenous injection of MSCs [17,18]. While local injection leads to better cell engraftment, cell viability could be limited due to the host microenvironment, particularly in the case of radiotherapy. After colorectal irradiation, the secretion of pro-inflammatory cytokines as IL1b, TGFb and chemoattractant has been demonstrated, as well as the huge infiltrate of innate immune cells [19,20]. In order to overcome these drawbacks, the use of a hydrogel that entraps the MSCs may protect the cells from the host tissue environment. The cytoprotective properties of the hydrogel could maintain the viability and function of the cells. This biomaterial-assisted cell therapy may make it possible to significantly increase the therapeutic benefit with a reduced number of injections. Indeed, in cases of ischemia (heart and hindlimb), studies have demonstrated that injection of MSCs embedded in biomaterials enhances their therapeutic benefit [21,22]. This method of injection is also used for articular cartilage repair by direct implantation with minimally invasive surgery [23].

Amongst the wide variety of biomaterials, biocompatible hydrogels may represent an excellent cell delivery system in view of their distinctive property of permitting *in-situ* gelation. Two types of hydrogels exist: natural and synthetic. Natural hydrogels are used as scaffolds because they display numerous biological functions that synthetic polymers lack, such as cell adhesion and biodegradation. A hybrid semi-synthetic, semi-biological silanized hydroxypropylmethyl cellulose (Si-HPMC) hydrogel has been designed as an injectable cell carrier. This Si-HPMC hydrogel is biocompatible, able to self-crosslink *in situ* to form a scaffold and maintain MSC phenotype, viability and secretion ability [23]. Indeed, Mathieu et al. have demonstrated the beneficial effect of Si-HPMC hydrogel in cardiac tissue engineering with a pronounced improvement in cardiac capacity after infarction when MSCs were injected within the biomaterial [22].

Here, we aimed to use a Si-HPMC hydrogel with rheological properties compatible with clinical use through a specific colonoscope catheter. We evaluated the effects of hydrogel-assisted Ad-MSC therapy compared with conventional intravenous injections of MSCs as used in clinical applications on structural and functional damage induced on a rat model of colorectal irradiation [4]. We also followed the engraftment of the Ad-MSCs and their fate in the lesion site. Lastly, we studied the hydrogel's ability to protect the cells from the deleterious irradiated environment.

2. Materials and methods

2.1. Ethics statement

All experiments were performed in compliance with the Guide for the Care and Use of Laboratory Animals as published by the French regulations for animal experiments (Ministry of Agriculture Order No. B92-032-01, 2006) with European Directives (86/609/CEE) and were approved by local ethical committee of the institute (permit number P13-14).

2.2. Animals and irradiation protocol

Male SD (Sprague Dawley, non-consanguineous) rats (Janvier SA, Le Genest St Isle, France) weighing 250 g were received and housed in a temperature-controlled room (21 ± 1 °C). They were allowed free access to water and fed standard pellets. Rats were anesthetized by isoflurane inhalation and a single 29 Gray (Gy) dose was delivered by a medical accelerator (Alphée) through a 2 × 3 cm window centered on the colorectal region. Alphée is an accelerator-type radiation source (maximal energy is 4 MeV with an average energy of about 1.5 MeV; 30 kA).

2.3. Cell culture and characterization

Adipose-derived MSCs were obtained by digesting the subcutaneous inguinal adipose tissue of seven-week-old GFP-transgenic SD rats as previously described [5]. After 7 days, the monolayer of adherent cells was trypsinized, washed in PBS three times before injection in rats. The phenotype of amplified Ad-MSC was verified by flow cytometry. The percentage of CD90 (clone OX-7; BD Biosciences, Le pont de Claix, France) and CD73 (clone 5F/B9; BD Biosciences) positive cells were analyzed and the absence of hematopoietic cells was verified with CD34 (clone ICO115, Santa Cruz, Dallas, Texas, USA) and CD45 (clone OX-1; BD Biosciences) markers. Isotype identical antibodies served as controls. On average, Ad-MSC expressed CD90 at 95.25% (±2.7), CD73 at 65.42% (±18.9), CD34 at 1.45% (±1.01) and CD45 at 0.575% (±0.2). The potential of adipogenic and osteogenic differentiations was also evaluated.

2.4. Hydrogel preparation

Hydroxypropyl methyl cellulose (Methocel® E4M Premium procured from Colorcon) was silanized by grafting 3-glycidoxypropyltrimethoxysilane GPTMS as previously described