

Abstract Presentation

Use of stem cells and autologous growth factors for the treatment of burns

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PURPOSE OF RESEARCH: In recent years, regenerative medicine has offered new therapeutic strategy to treat the burns. Recent studies have shown clinical efficacy in advanced therapies involving the combined use of autologous cells and growth factors. The advantage of these therapies is based on the possibility of exploiting the synergy between the regenerative power of growth factors and stimulation the of proliferation of stem cells. In our Center we have implemented an experimental trial in order to evaluate the clinical efficacy of combination therapy directed to lifesaving treatment of burns, based on the preparation of autologous platelet gel in combination with autologous mesenchymal stem cells of bone marrow origin and / or adult stem cells (keratinocytes / fibroblasts).

METHODS: Consistent with the regulations the autologous platelet gel was prepared in collaboration with the Transfusional Medicine, using a system of tubes certificate (MyCells). The autologous PRP patients obtained with this system was associated with stem cells, not expanded in culture and characterized biologically previously.

RESULTS AND CONCLUSIONS: The authors present the most representative clinical cases of severely burned patients treated topically with this therapeutic approach wich were not responding to the conventional treatments. We also compare of the different methods of preparation of stem cells, the one of mesenchymal bone marrow origin and the other origin of skin, highlighting the limits and benefits of different working methods. The results of early follow-up on patients at 15 days after treatment showed a complete regeneration of skin tissue, characterized by the presence of histological islands of skin around the area of the lesion treated. Subsequent follow-up after 30 days of treatment demonstrated the presence of a more mature skin tissue largely packed with numerous cells. The results obtained to date encourage us to continue the study identifyng as next target the search for a possible correlation between biological dose and clinical efficacy in burns treatment.

Human neural stem cell (hNSCs) characterization in good manufacturing practice production (GMP)

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Irrespective of their specific etiology, neurodegenerative disorders eventually lead to loss or functional alteration of mature cells of the brain parenchyma. Symptoms vary significantly, depending on many parameters, such as age of onset, region of the brain the type of cells being damaged and the origin and nature of the *noxa* – genetic, toxic, traumatic, ischemic, hemorrhagic, infectious or immunological and inflammatory, to highlight the major examples.

Given this scenario, it is immediately clear how the translation of any experimental, neural stem cell-based transplantation approach into clinical protocols, vitally hinges on the establishment and validation of a reliable and renewable source of hNSCs.

In the last few years we improved a laboratoristic method to produce human neural stem cells (hNSCs) under GMP conditions. These guidelines initially studied to rule pharmaceutical productions impose the drug characterization and the improvement of tests to assure the safety during the use in humans. We decided to produce hNSCs cause these cells represent a potential therapeutic tool for neurodegenerative diseases thanks to their peculiar characteristics like differentiation potentiality, self renewal, plasticity and the ability to integrate into hosts tissues and produce several growth factors and cytokines. Moreover, several recent studies have demonstrated that these cells are able to improve the clinical condition in animals model with diseases involving the central nervous system like ALS, multiple sclerosis or stroke.

We found that our neural cell lines derived from human central nervous system, cultured in serum free medium containing EGF and bFGF, generated growth curves stable up to several passages maintaining stable genetic assessment. Moreover the cells have shown the ability to differenziare in neurons, astrocytes and oligodendrocytes the main central nervous system cells. The clonogenic assay showed the hNSC clonal ability one of stemness characteristic.

Also our cells cultured with a medium devoid of growth factors are met with cell death showing that growth conditions do not transform cells

Preliminary results on transplantation in animal models show hNSC migration and integration without tumor development.

We present the result of this process of certification for a sample of these GMP-grade hNSCs lines.

Triggering osteogenesis in adult and induced pluripotent stem cells by nanocomposite scaffold

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Aim. Tissue engineering has become a rapidly expanding research area since it offers a promising approach for tissues repair and regeneration. Bone engineering represents an ideal model to investigate how stem cells can act as mechanosensitive units responding to the mechanical stimulation of the extracellular matrix through focal adhesions and changes in cytoskeletal organization.

We have recently fabricated a PLLA and PLLA/d-Hap scaffolds by electrospinning filled with hydroxyapatite at several concentrations (1wt.% to 8 wt.%).

In this study we asked whether each 3-dimensional scaffold drives the osteogenic differentiation of adult stem cells and if this effect is stem cell type specific.

To address the first question we cultured human bone marrow mesenchymal stem cells (hBM-MSCs) on PLLA/1d-Hap, PLLA/8d-Hap and PLLA scaffolds in the presence/absence of osteogenic medium. Results. Our results demonstrated that PLLA/d-Hap nanocomposite scaffolds, even in the absence of osteogenic medium, induce hBM-MSCs to differentiate towards the osteocytes.

We get insights to the second issue exploring the effect of these scaffolds in murine induced pluripotent stem (iPS) cells. As references similar experiments were conducted using murine embryonic stem (ES) cells.

We found that the PLLA/d-Hap scaffolds supported the osteogenic differentiation of iPS and ES cells in vitro even in the absence of osteogenic inducers.

The overall data demonstrated that the PLLA/d-HAp scaffolds have a strong osteoinductive activity which is independent of the stem cell types. Thus, our work proposes PLLA/d-HAp for bone engineering applications without administration of osteogenic inducers.

Acknowledgments

This study was supported by the Italian Fondazione Cassa di Risparmio di Perugia (grant no. 2009.020.0050 to A.O.), the Italian Ministero dell'Istruzione, dell'Università e della Ricerca (grants: FIRB Idea Progettuale no. RBIP06FH7J_002 and PRIN no. 20084XRSBS_001 to A.O.), as well as the Istituto Nazionale Biostrutture e Biosistemi.

Omentum-derived stromal cells improve myocardial regeneration in pig post-infarcted heart through a potent paracrine mechanism

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Introduction: Myocardial Infarction (MI) represents one of principal causes of human death. Cell-based therapy could be a valid new approach for MI. Different kind of cells, including embryonic stem cells as well as adult/progenitor stem cells, have been proposed as candidates for therapeutic purposes. However, many aspects, ranging from ethical questions to functional efficacy, still remain to be clarified. Among adult/progenitor stem cells, Adipose-derived stromal cells (ADSC) seem to have some advantages, mainly because of their easy tissue accessibility and in vitro an adequate rate of growth.

Aim of the study: To investigate the capacity of transplanted ADSCs, through functional, haemodynamic and histopathological assessment, to improve myocardial infarction and regeneration of experimental heart ischemia induced by permanent IVA-ligation in pigs.

Methods: ADSCs isolated from human adipose tissue (omentum fat) were cultured, expanded, and phenotypically characterized. Furthermore, in vitro proangiogenic, anti-inflammatory and anti-apoptotic properties were analyzed. 50x10⁶ cells/pig were transplanted by intramyocardial injection in acute infarcted hearts (treated-group, n=12 cell-injected pigs). Two months after MI induction echocardiographic and haemodynamic follow-up was performed. In addition, histopathological examination was conducted.

Results: in vitro ADSCs secreted high levels of pro-angiogenic, anti-inflammatory and immunomodulatory cytokines (VEGF, HGF and IL-6).

Furthermore, they prevented monocytes activation as well as cardiomyocytes apoptosis. Finally, in vitro but not in vivo, ADSCs were able to transdifferentiate into cardiomyocyte-like cells. In vivo, ADSCs injection along the border of the ischemic area, reduced post-infarct pigs

mortality, produced a significant ameliorative effect on heart haemodynamic parameters and slightly improved echocardiographic profile. Histological and immunohistochemical examination demonstrated some cardio-regenerative capacities of ADSCs, showing an increase of vascular and cardiomyocyte markers only in animals treated with ADSCs.

Conclusions: Implanted ADSCs derived from omentum could improve myocardial function and regeneration through the concomitant capacity to release molecules, restore angiogenesis, reduce inflammation and prevent cardiomyocytes apoptosis. Since adipose tissue is one of the body's richest known sources of regenerative cells, ADSCs could play a critical role in limiting or reversing heart damage caused by a heart attack.