Cytotherapy, 2014; 0: 1–14

International Society for Cellular Therapy
ISCT®

Inactivated human platelet lysate with psoralen: a new perspective for mesenchymal stromal cell production in good manufacturing practice conditions

SARA CASTIGLIA[1,*], KATIA MARESCHI[1,2,*], LUCIANA LABANCA[3], GRAZIELLA LUCANIA[3], MARCO LEONE[1], FIORELLA SANAVIO[1], LAURA CASTELLO[1], DEBORAH RUSTICHELLI[1], ELENA SIGNORINO[1], MONICA GUNETTI[1], MASSIMILIANO BERGALLO[2], ANNA MARIA BORDIGA[3], IVANA FERRERO[1,2] & FRANCA FAGIOLI[1]

[1]*Pediatric Onco-Hematology, Stem Cell Transplantation and Cellular Therapy Division, City of Science and Health of Turin, Regina Margherita Children's Hospital, Turin, Italy,* [2]*Department of Public Health and Pediatrics, University of Turin, Italy, and* [3]*Blood Component Production and Validation Center, City of Science and Health of Turin, S. Anna Hospital, Turin, Italy*

Abstract

Background aims. Mesenchymal stromal cells (MSC) are ideal candidates for regenerative and immunomodulatory therapies. The use of xenogeneic protein–free good manufacturing practice–compliant growth media is a prerequisite for clinical MSC isolation and expansion. Human platelet lysate (HPL) has been efficiently implemented into MSC clinical manufacturing as a substitute for fetal bovine serum (FBS). Because the use of human-derived blood materials alleviates immunologic risks but not the transmission of blood-borne viruses, the aim of our study was to test an even safer alternative than HPL to FBS: HPL subjected to pathogen inactivation by psoralen (iHPL). *Methods.* Bone marrow samples were plated and expanded in α-minimum essential medium with 10% of three culture supplements: HPL, iHPL and FBS, at the same time. MSC morphology, growth and immunophenotype were analyzed at each passage. Karyotype, tumorigenicity and sterility were analyzed at the third passage. Statistical analyses were performed. *Results.* The MSCs cultivated in the three different culture conditions showed no significant differences in terms of fibroblast colony-forming unit number, immunophenotype or in their multipotent capacity. Conversely, the HPL/iHPL-MSCs were smaller, more numerous, had a higher proliferative potential and showed a higher Oct-3/4 and NANOG protein expression than did FBS-MSCs. Although HPL/iHPL-MSCs exhibit characteristics that may be attributable to a higher primitive stemness than FBS-MSCs, no tumorigenic mutations or karyotype modifications were observed. *Conclusions.* We demonstrated that iHPL is safer than HPL and represents a good, good manufacturing practice–compliant alternative to FBS for MSC clinical production that is even more advantageous in terms of cellular growth and stemness.

Key Words: *Good Manufacturing Practice, human platelet lysate, inactivation, mesenchymal stromal cells, psoralen*

Introduction

Rapid progress in the fields of biotechnology and medicine has led to the development of new treatments and innovative medicinal products. Among the latter, new cell-based medicinal products, containing viable human cells of autologous or allogeneic origin, have a high potential for cell-based therapies for various severe diseases. In particular, mesenchymal stromal cells (MSCs) can easily be isolated from bone marrow (BM), thanks to their capacity to adhere and proliferate and expand in culture (1,2). MSCs are multipotent stem cells with high immunomodulant proprieties, and they produce multiple cytokines, growth factors and adhesion molecules, all important factors that influence the hematopoietic microenvironment (3,4). These cells' particular characteristics and high plasticity make them relevant in the fields of cell therapy, tissue repair and in tissue engineering strategies as therapeutic products tailored to a

*These authors contributed equally to this work.
Correspondence: **Katia Mareschi**, ScB, Pediatric Onco-Hematology, Stem Cell Transplantation and Cellular Therapy Division, City of Science and Health of Turin, Regina Margherita Children's Hospital, Department of Public Health and Pediatrics, University of Turin, Italy, Piazza Polonia, 94 10126 Turin, Italy.
E-mail: katia.mareschi@unito.it

(Received 25 June 2013; accepted 22 December 2013)

ISSN 1465-3249
http://dx.doi.org/10.1016/j.jcyt.2013.12.008

number of clinical scenarios, from degenerative to post-traumatic diseases caused by damage or cell loss (5,6). The increasing use of MSCs as advanced therapy medicinal products (ATMP) has led to production processes that need to meet good manufacturing practice (GMP) (7,8).

The regulatory context for advanced therapy medicinal products is set out in Regulation (EC) N. 1394/2007, which is designed to facilitate patient access to these products while guaranteeing the highest level of safety for patients (9).

To ensure product safety and efficacy, GMP guarantees that products are consistently produced and controlled at the quality standards required for their intended use, from collection to release, including cell harvesting, cell manipulation processes, the maximum number of cell passages, combination with other components of the product, filling, packaging and so forth. Although human MSCs are not highly immunogenic, when expanded in xenogeneic sera such as in fetal bovine serum (FBS), they are likely to generate immune responses in some patients after administration (10,11). It has been shown that a single preparation of 10^8 human MSCs grown under standard conditions in FBS carries with it approximately 7–30 mg of FBS proteins (10). Thus, in view of clinical GMP production, the use of xenogenic serum is complicated because there is high lot-to-lot variability and it is associated with a risk of transmitting infectious agents and immunizing effects (12,13).

Therefore, regulatory guidelines for GMP production, aimed at minimizing the use of FBS, used in most expansion protocols as a cell culturing medium supplement, have further reinforced intensive searches for safer media supplementation alternatives (14).

Interesting studies have evidenced the possibility of replacing FBS with autologous or allogeneic platelet lysate obtained from single-donor or pooled human serum or from platelets because they contain a plethora of growth-promoting factors. Moreover, human platelet lysate (HPL) is being established as a safe and efficient MSC culture supplement for robust MSC cultivation, thus offering certain advantages as a potential FBS substitute (15–18). Although the use of human-derived blood materials alleviates the immunologic risks of FBS, the possibility of transmitting blood-borne viruses remains, especially when materials from multiple donors are pooled to provide a sufficient volume for therapeutic-scale MSC expansion and to limit individual donor variability. Pathogen inactivation (PI) technologies are aimed at enhancing blood safety through the inactivation of emerging pathogens, both known and as yet-unidentified ones that and not detected by current screening or testing protocols. Since 1990, significant progress has been made in pathogen inactivation technology, which at present is widely available in European blood services, with multiple CE (an abbreviation of Conformité Européenne, ie, "European Conformity") marked products to treat both platelets and plasma for transfusion. The CE marking states that a product has been assessed before being placed on the market and meets EU safety, health and environmental protection requirements.

The European experience with pathogen-inactivated platelets and plasma now numbers millions of units, with a safety record that has been widely reported in the literature (19) and extensive validation studies, through the use of blood products with high titers of added bacteria, enveloped and non-enveloped viruses and protozoa, have been performed to prove their efficacy.

Therefore, the application of the PI technique on platelets to be used for the preparation of HPL to supplement culture medium for MSC expansion in a GMP setting appears to be highly desirable because it might obviate virus transmission problems.

In the present study, the PI process was performed with the use of Intercept Blood System technology (INTERCEPT; Cerus Europe BV, Amersfoort, The Netherlands), which uses a photoactive compound, a derivative of psoralen (Amotosalem) and long-wavelength ultraviolet (UVA) illumination. On exposure to UVA light, Amotosalem becomes reactive and forms a chemical cross-link that locksup the strands of RNA and DNA, blocking and inactivating the replication of viruses, bacteria and leukocytes in platelet concentrates (20–22).

In our study, to ensure that the inactivation process does not induce changes in the cells, we set up, in parallel, MSC cultures in FBS (the standard supplement), HPL (recently used in GMP conditions as a cell growth supplement) and iHPL (the same batch of HPL after inactivation). We then compared the effects of the three supplements on cell growth, immunophenotype, multipotent capacity, karyotype, tumorigenesis and stemness protein expression.

Methods

HPL preparation

Whole blood (450 ± 45 mL) collection from 60 healthy blood donors was performed in a triple-bag system (Fresenius Kabi, Bad Homburg, Germany) containing 63 mL of citrate-phosphate-dextrose as an anticoagulant. According to Italian laws and European guidelines (19), the routine testing of blood donors was performed for the following: ABO blood groups, irregular red blood cell antibodies and