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Good manufacturing practice-compliant validation and preparation of BM cells for the therapy of acute myocardial infarction

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Background

Intracoronary application of BM-derived cells for the treatment of acute myocardial infarction (AMI) is currently being studied intensively. Simultaneously, strict legal requirements surround the production of cells for clinical studies. Thus good manufacturing practice (GMP)-compliant collection and preparation of BM for patients with AMI was established by the Cytonet group.

Methods

*As well as fulfillment of standard GMP requirements, including a manufacturing license, validation of the preparation process and the final product was performed. Whole blood (*n = *6) and BM (*n = *3) validation samples were processed under GMP conditions by gelafundin or hydroxyethylstarch sedimentation in order to reduce erythrocytes/platelets and volume and to achieve specifications defined in advance. Special attention was paid to the free potassium (*< 6 *mmol/L), some rheologically relevant cellular characteristics (hematocrit* < 0.45*, platelets* $< 450 \times 10^6$*/mL) and the sterility of the final product.*

Results

The data were reviewed and GMP compliance was confirmed by the German authorities (Paul-Ehrlich Institute). Forty-five BM cell preparations for clinical use were carried out following the validated methodology and standards. Additionally three selections of CD34$^+$ BM cells for infusion were performed. All specification limits were met.

Discussion

In conclusion, preparation of BM cells for intracoronary application is feasible under GMP conditions. As the results of sterility testing may not be available at the time of intracoronary application, the highest possible standards to avoid bacterial and other contaminations have to be applied. The increased expense of the GMP-compliant process can be justified by higher safety for patients and better control of the final product.

Keywords

acute myocardial infarction, BM nucleated cells, CD34+ cells, cell therapy, cellular therapeutics, good manufacturing practice, production validation.

Introduction

Cell therapy for acute myocardial infarction (AMI) represents one of the emerging fields in experimental and clinical cardiology [1–5]. Animal and *in vitro* studies suggest that transfer of stem and progenitor cells can improve tissue perfusion and contractile performance of the infarcted heart [2,5–12]. Although the mechanisms promoting cardiac repair after cell transfer are not understood, stable cell engraftment and transdifferentiation of transplanted cells into cardiomyocytes and/or endothelial cell lineages and the release of paracrine mediators have been proposed as potential explanations [13,14]. Initial clinical efficacy data indicate that stem cells have the potential to enhance myocardial contractile performance

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in patients with AMI [11,15–19]. Autologous native (or minimal manipulated) BM-derived nucleated cells (BM-NC) and isolated mononuclear cells (BM-MNC), BM-derived mesenchymal stromal cells, BM-derived $CD133^+$ cells and circulating progenitor cells (CPC) have been used in these studies [5,8,9,12,15,20–22].

The responsible authorities in Europe and the USA (FDA, EMEA, ICH and national European authorities) strive to prepare regulations for the production of cellular therapeutics in compliance with the current rules of state-of-the-art drug manufacturing and according to specific scientific issues in the field. The corresponding professional and scientific societies (ISCT, AABB, EBMT, etc.) are heavily involved in these legal processes and are currently establishing standards and accreditation procedures to be followed (FACT and JACIE). Authorities agree that the production of cellular therapeutics should be carried out at least according to the good tissue practice (GTP) regulations [23,24]. In addition, stringent regulations for conducting of clinical studies have been executed [25,26]. In Germany, however, BM and all other hematopoietic preparations are currently stated as drugs (medicinal products) and thus fall completely under the requirements of good manufacturing practice (GMP) [27].

In this legal context, Cytonet Hannover GmbH (Hannover) has prepared and delivered BM-derived cells according to EU GMP guidelines and its pharmaceutical production license, for the BM transfer to enhance ST-elevation infarct regeneration (BOOST) trial that was conducted at the Hannover Medical School. The data presented here were obtained during the BOOST trial and an additional clinical study looking at the homing of BM-derived cells after intracoronary delivery [15,28]. The validation strategy and the handling of this validation, as well as the results of the production of BM-derived cells for the treatment of patients with AMI within the BOOST trial, are presented below.

Methods

Legal assumptions

As an owner of a production license for the preparation of BM and other hematopoietic cell grafts, including $CD34^+$ cell selections, Cytonet carries out its pharmaceutical production activities according to the German drug law and EU GMP guidelines [26,27]. The quality and safety of the cell preparations (cell therapeutics, medicinal products) are ensured through administration of a comprehensive quality management system. The organization structure, qualification and training status of the personnel, and the appropriately designed, installed and maintained premises and equipment, are compliant with current EU GMP standards. All records are kept as required by good documentation practice. Every production and quality control procedure, including contract manufacturing and analyzes, are kept permanently in a validated status and are revalidated on an annual basis or when necessary (major changes). Complaints and product recalls are promptly handled. An internal control of all systems is ensured by regular self-inspections and audits. All work is carried out according to written standard operating procedures.

Because BM preparation includes handling of 'open products' while connecting transfer bags, filters, syringes and buffers and taking test samples, the corresponding steps are performed in a processing facility of class A laminar air-flow hoods placed in class B clean rooms, as demanded by the current EU GMP guidelines (refer to Annex 1 of the guidelines). The final product has to be infused intracoronarily immediately and before all results of sterility testing become available, thus appropriate 'on-line' personnel and working area monitoring and sterility testing are carried out. Media fills are performed on a regular basis in order to control critical production steps. All patients give their written informed consent for the described procedures following approval by the local ethical committee at the Hannover Medical School.

Presented data

The data from three different and relatively independent experiment groups are presented below. The first data pool describes the validation of the BM preparation, by using whole blood as dummy material, carried out by the Cytonet Hannover GmbH. The BM used for infusion within the Hannover BOOST trial was manufactured in the same facility and by using exactly the same manufacturing strategy (second data pool). Within another validation study, which is shown as third separate third data pool, another Cytonet production site (Cytonet Heidelberg GmbH) used BM and a different sedimentation reagent (results are shown as supporting data). The differences of the manufacturing processes between Hannover and Heidelberg are described separately or pointed out in the text.