Myocardial regeneration strategy using Wharton's jelly mesenchymal stem cells as an off-the-shelf 'unlimited' therapeutic agent: results from the Acute Myocardial Infarction First-in-Man Study

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Abstract

Introduction: In large-animal acute myocardial infarction (AMI) models, Wharton's jelly (umbilical cord matrix) mesenchymal stem cells (WJMSCs) effectively promote angiogenesis and drive functional myocardial regeneration. Human data are lacking.

Aim: To evaluate the feasibility and safety of a novel myocardial regeneration strategy using human WJMSCs as a unique, allogenic but immuno-privileged, off-the-shelf cellular therapeutic agent.

Material and methods: The inclusion criterion was first, large (LVEF ≤ 45%, CK-MB > 100 U/l) AMI with successful infarct-related artery primary percutaneous coronary intervention reperfusion (TIMI ≥ 2). Ten consecutive patients (age 32–65 years, peak hs-troponin T 17.3 ±9.1 ng/ml and peak CK-MB 533 ±89 U/l, sustained echo LVEF reduction to 37.6 ±2.6%, cMRI LVEF 40.3 ±2.7% and infarct size 20.1 ±2.8%) were enrolled.

Results: 30 × 10^6 WJMSCs were administered (LAD/Cx/RCA in 6/3/1) per protocol at ≈ 5–7 days using a cell delivery-dedicated, coronary-non-occlusive method. No clinical symptoms or ECG signs of myocardial ischemia occurred. There was no epicardial flow or myocardial perfusion impairment (TIMI-3 in all; cTFC 45 ±8 vs. 44 ±9, $p = 0.51$), and no patient showed hs-troponin T elevation (0.92 ±0.29 ≤ 24 h before vs. 0.89 ±0.28 ≤ 24 h after; decrease, $p = 0.04$). One subject experienced, 2 days after cell transfer, a transient temperature rise (38.9°C); this was reactive to paracetamol with no sequel. No other adverse events and no significant arrhythmias (ECG Holter) occurred. Up to 12 months there was one new, non-index territory lethal AMI but no adverse events that might be attributable to WJMSC treatment.

Conclusions: This study demonstrated the feasibility and procedural safety of WJMSC use as off-the-shelf cellular therapy in human AMI and suggested further clinical safety of WJMSC cardiac transfer, providing a basis for randomized placebo-controlled endpoint-powered evaluation.

Key words: myocardial regeneration, Wharton's jelly mesenchymal stem cells, human umbilical cord matrix, acute myocardial infarction, first-in-man, safety, feasibility.

Introduction

Current cellular treatment strategies aimed to stimulate cardiac regeneration in human acute myocardial infarction (AMI) suffer from lack of highly potent therapeutic cells available *ad hoc* in sufficient numbers [1].

Wharton's jelly mesenchymal stem cells (WJMSCs) are a small population of multipotent progenitor cells naturally present in the human umbilical cord matrix [2]. In the ongoing search for the best stem cell to stimulate myocardial regeneration in AMI [3], WJMSCs are an

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attractive candidate [4] for a readily available "off-the-shelf" cellular regenerative product for human use for several reasons. First, the umbilical cord is one of the most easily reached stem cell sources in the absence of ethical concerns associated with using embryo-derived stem cells [5, 6]. Secondly, WJMSCs spontaneously secrete numerous pro-angiogenic factors, such as vascular endothelial growth factor (VEGF), angiopoietin-1, HGF and transforming growth factor β1 (TGF-β1) [7], and the release of pro-angiogenic molecules is accompanied by evidence of functional angiogenic potency in the infarcted mammalian heart [7–10]. Furthermore, WJMSCs show spontaneous expression of a number of early cardiac transcription factors in addition to their natural expression of core markers of undifferentiated human embryonic stem cells [11]. Wharton's jelly mesenchymal stem cells are more efficient in terms of stem cell potency than, for instance, bone marrow-derived stem cells predominantly used to date [5].

Wharton's jelly mesenchymal stem cells are naturally chemoattracted to the cardiac tissue, and they possess functional ability to populate the ventricular myocardium [12]. Indeed, in animal models, WJMSCs effectively induce angiogenesis [8–10] and show spontaneous differentiation into cardiac myocytes and endothelial cells [8, 9, 13], a process consistent with the early findings in cardiomyocyte-conditioned medium WJMSC cultures [2]. In addition, recent evidence shows that WJMSCs can be successfully expanded *ex vivo* with high genomic stability, full clonogenic potential and full multilineage differentiation capacity, and with fully functional angiogenic potency, yielding large quantities of therapeutic cells available "when needed" and in "as-needed" numbers [7].

Wharton's jelly mesenchymal stem cells use is free of the logistic problems of autologous cell harvest in clinically unstable patients and circumvents the problem of the generally low (and variable) autologous regenerative cell yields [14, 15]. This may be particularly relevant in AMI, when the biologic window for therapeutic cell delivery does not allow any long-term culture and/or cell manipulation or lineage-specific expansion of autologous cells [16]. Although WJMSCs are a natural candidate for application in the setting of AMI in man, human feasibility and safety data are lacking [17, 18] (NB. PubMed exploration using various combinations of the terms "Wharton's jelly, umbilical cord matrix, infarction, heart, cardiac, myocardial, clinical, experimental, man or human"; last search performed on 20 May 2015).

Aim

The aim of the present work was to evaluate the feasibility and safety of a novel myocardial regeneration strategy using WJMSCs as an off-the-shelf 'unlimited' therapeutic agent.

Material and methods

Patient enrollment, infarct size data and cardiac imaging

Consecutive patients with successful infarct-related artery (IRA) stent-assisted primary percutaneous coronary intervention (pPCI) reperfusion (TIMI ≥ 2) in large AMI, defined as sustained left ventricular ejection fraction (LVEF) reduction to ≤ 45% and peak creatine kinase-MB (CK-MB) level exceeding 100 U/l (upper limit of normal (ULN) < 24 U/l), were screened for enrollment as described in our earlier protocols [14, 15] except that the present study group was not limited to left anterior descending coronary artery (LAD) infarcts. Also, in the present study patients with two-vessel disease could be enrolled, but full coronary revascularization was required prior to cell transfer. No patients with three-vessel disease were recruited. The study was compliant with the Declaration of Helsinki and it was approved by the local Ethics Committee. All subjects provided informed written consent.

Echocardiography, gadolinium-enhanced cardiac magnetic resonance imaging (cMRI) and ECG-gated single photon emission computed tomography (SPECT) were performed within 24–48 h prior to cell transfer as described in our prior work [15].

Human WJMSCs harvest and expansion

The cells were harvested and prepared as described previously [2, 5, 7, 10]. Umbilical donations were obtained following informed written consent, consistent with Directive 2004/23/EC, which sets the standards of quality and safety for the donation, procurement, testing, processing, preservation, storage, and distribution of human tissues and cells. Cell preparation was consistent with the International Conference on Harmonization and the US Food and Drug Administration (FDA) guidelines on stem cell-based therapies [19]. All solutions were prepared under Good Manufacturing Practice and all procedures were performed in line with Good Laboratory Practice.

In brief, MSCs were isolated using an enzymatic digestion strategy. Isolated cells were plated into 75 cm^2 tissue culture flasks (Sarstedt Stare Babice, Poland) in Dulbecco's modified Eagle's medium (PAA Laboratories GmbH, Goetzis, Austria) supplemented with 10% FBS (PAA Laboratories GmbH, Goetzis, Austria), PDGF (PeproTech Inc, Rocky Hill, USA) and EGF (PeproTech Inc, Rocky Hill, USA) growth factors. Flasks were incubated at 37°C in a humidified atmosphere containing 5% CO_2, and after 7 days the medium was changed. At confluence, the adherent cells were detached using 0.25% trypsin and re-seeded at 0.075 × 10^6 cells/75 cm^2 flask and incubated again until confluence with a weekly medium change. After the second passage, cells were collected and frozen in 2.5 × 10^6 aliquots in liquid nitrogen. Five–seven days

before treatment cells were thawed and expanded to obtain at least 30 × 10^6 cells.

Wharton's jelly mesenchymal stem cells preparation for human application

Phenotypic evaluation of cultured WJMSCs

The MSC phenotype was analyzed with antibodies specific for MSCs (CD73, CD90, and CD105) and hematopoietic cells (CD45) (all antibodies from BD Biosciences, San Jose, USA) using a FACSCalibur cytometer with CellQuest software (BD Biosciences Immunocytometry Systems, San Jose, USA). Wharton's jelly mesenchymal stem cells used for transplantation were ≥ 95% positive for CD73, CD90, CD105 and negative for CD45, and their proliferative and functional capacity was evaluated.

Evaluation of cytogenetic stability of cultured WJMSCs

Cytogenetic stability of cultured WJMSCs was confirmed via GTG banding. Metaphases were analyzed under an Olympus BX51 microscope with a camera (Olympus Corporation, Tokyo, Japan) to document photomicrographs. The CytoVision program (Leica Microsystems Inc., Buffalo Grove, USA) was used to arrange chromosomes into a karyogram.

Sterility evaluation and obtaining single-cell suspension

Sterility of the material was confirmed via microbiological evaluation. Immediately prior to transfer to the cathlab, WJMSCs were filtered to eliminate any potential cellular conglomerates that might cause microvascular plugging [20] and were suspended in 2 ml of 0.9% NaCl (B. Braun, Melsungen, Germany). In addition, the cells were gently tottered throughout the transport period to minimize any formation of cellular aggregates.

Trans-IRA cell administration

Cell administration to the infarct zone was performed using the previously described non-coronary occlusive technique for transcoronary delivery of cellular therapies [14, 21, 22]. 30 × 10^6 WJMSCs were re-suspended in 30 ml of 0.9% saline and were administered via the IRA using a cell-delivery perfusion catheter [14, 15], using a filter to eliminate any potential cellular conglomerates. The cell suspension infusion was at 2 ml/min, yielding 2 × 10^6 cells per minute.

Clinical, angiographic and cardionecrotic marker evaluation

Throughout the cell transfer, ECG was monitored carefully for any new ischemic changes or arrhythmias [14, 21]. Angiographic recordings were performed to evaluate the coronary epicardial flow and myocardial tissue perfusion using the established indices (TIMI grade, corrected TIMI frame count, cTFC) [23]. Hs-troponin T level (ULN < 0.014 ng/ml) was determined ≤ 24 h before and ≤ 24 h after cell transfer.

In-hospital follow-up and post-discharge patient monitoring

Twenty-four hours ECG recordings were performed 24–48 h after cell administration. Close clinical vigilance has been applied for any adverse events up to 12 months (with further pre-specified points of analysis at every 12 months up to 5 years). A study-independent Safety Committee at the Jagiellonian University Department of Cardiac and Vascular Diseases performed initial event analysis in the context of the likelihood of the event association with cell transfer, and it oversaw any potential EC notification(s).

This work is consistent with the US Food and Drug Administration (FDA) guidelines on stem cell-based therapies [19].

Statistical analysis

Data are shown as mean ± SEM (min.-max.) or number (%). Two-tailed Student *t*-test was used for statistical comparisons (Statistica 10.0, StatSoft).

Results

Clinical characteristics of study patients and infarct size data are shown in Table I, indicating recruitment of a typical group of consecutive patients with a large, first acute myocardial infarction.

Except for the WJMSC transfer using a transradial approach at ≈ 5–7 days after pPCI, patient management was standard and was consistent with the European Society of Cardiology (ESC) guidelines. Myocardial imaging with SPECT and cMRI [15] was uncomplicated. No clinical complications occurred in the study group by the point of cell transfer.

No WJMSC genetic instability was detected and no or other laboratory-level safety issues occurred. Wharton's jelly mesenchymal stem cells transfer was performed per protocol in all study patients. No clinical symptoms or signs of myocardial ischaemia, and no new ischemic ECG changes occurred during or after WJMSC trans-IRA administration.

There was no indication of any negative effect of trans-IRA WJMSC transfer on epicardial or myocardial perfusion. In particular, the epicardial flow was TIMI-3 in all subjects before and after cell transfer, and cTFC was 45 ±8 (from 17 to 76) prior to cell transfer and 44 ±9 (from 15 to 77) after cell transfer (p = 0.51). Also, no patient showed a hs-troponin T rise after cell transcoronary WJMSC transfer. The mean hs-troponin T level was 0.92 ±0.29 (from 0.04 to 2.64) within 24 h before vs. 0.89

±0.28 (from 0.03 to 2.52 ng/ml) within 24 h after cell administration (p = 0.04 for hs-troponin T decrease).

One subject experienced, two days after cell transfer, a transient temperature rise to 38.9°C; this was reactive to paracetamol, with no signs of infection and no clinical sequel. The association between this event and cell transfer was considered possible, although transient fever may occur in the AMI recovery phase without any apparent cause other than myocardial necrosis [24].

One other patient had vein inflammation at the site of venous line insertion that was managed in a typical manner, and another, treated empirically with amoxicillin due to chronic obstructive pulmonary disease infectious exacerbation, exhibited a typical picture of amoxicillin-associated skin rash that was responsive to steroid and antihistaminic administration and amoxicillin withdrawal. Any potential association between the two latter events and WJMSC administration was judged, at the level of the Safety Committee, to be highly unlikely.

All three events were reported to the local EC, and no reciprocal safety concern was raised. No other adverse events and no significant arrhythmias (ECG Holter) occurred.

At discharge, the patients were transferred to a routine post-AMI rehabilitation program. Up to 12 months there was one new, non-index territory AMI that led to death but no adverse events that might be attributable to WJMSC treatment.

In summary, the one and only adverse event that was considered potentially related to WJMSC administration was a transient temperature rise in 1 patient that was responsive to typical paracetamol treatment and resolved rapidly and without sequel.

Table I. Study group (n = 10) characteristics and myocardial infarction parameters

Variable	Mean ± SEM (min.-max.) or n (%)
Age [years]	55.6 ±3 (32–65)
Women	5 (50)
Diabetes	3 (30)
Prior MI	0 (0)
IRA = LAD/Cx/RCA	6/3/1 (60/30/10)
IIb/IIIa rec. inhibitor (Abciximab)	4 (40)
IRA DES/BVS	9/1 (90/10)
IRA TIMI-3/TIMI-2*	8/2 (80/20)
Peak hs-troponin T [ng/ml]	17.3 ±9.1 (5.2–102.1)
Peak CK-MB [U/l]	533 ±89 (148–965)
Echo LVEF§ [%]	37.6 ±2.6 (21–43)
MRI LVEF§ [%]	40.3 ±2.7 (26–49)
G-SPECT LVEF§ [%]	36.8 ±2.8 (19–48)
MRI infarct size# [%]	20.1 ±2.8 (7–41)
SPECT infarct size# [%]	33.7 ±3.1 (18–52)

*SEM – standard error of the mean, IRA – infarct-related artery, LAD/Cx/RCA – left anterior descending/circumflex/right coronary artery, LVEF – left ventricular ejection fraction, echo – echocardiography, SPECT – single photon emission computed tomography, G-SPECT = gated SPECT. *At pPCI completion (NB. at the cell transfer point all IRAs showed TIMI-3 flow), §≤ 24 h from cell transfer to minimize the effect of myocardial stunning on LVEF as an acute index of infarct size [15], #expressed as proportion of left ventricular muscle volume (MRI) or as infarct extent (SPECT).*

Discussion

The principal finding from this work is the feasibility and procedural safety of WJMSC use as an off-the-shelf, transcoronary cellular allogenic therapy in AMI in man. Our study also suggests further, post-procedural clinical safety of WJMSC cardiac transfer in humans, opening a new avenue towards controlled outcome studies using WJMSCs in AMI and in other conditions associated with depletion of functional myocardial tissue.

Regeneration therapies in the era of optimal pharmaco-mechanical AMI treatment: reversal of the damage as a logical step to follow maximized reduction of myocardial tissue loss

Several lines of evidence suggest that, in AMI, conventional pharmacological and mechanical therapies may have already reached the ceiling of efficacy (or are very close to such) in their capacity to limit myocardial injury and reduce the infarct size [25]. The concept of myocardial injury reversal (as the next step to follow maximized limitation as explored and implemented over the last three decades) by stimulating myocardial regeneration is gradually gaining evidence of feasibility in the experimental and clinical setting, and cellular therapies are a pillar of this concept [3]. Nevertheless – and disappointingly to those who have worked extensively to advance the field over the last years – the efficacy of cellular therapies today remains too modest for any systematic clinical application [1, 3, 26]. Apart from the room for further improvement of cell delivery strategies [14, 16], important limiting factors are (i) lack of identification of sufficiently potent and clinically safe cell types, and (ii) insufficient quantities of (in the majority of studies – autologous) regenerative cells and lack of their 'off-the-shelf' availability. Indeed, both experimental and clinical data indicate that the therapeutic effect of progenitor cells is dose (i.e. therapeutic cell number)-dependent [26–28]. Autologous non-expanded/engineered cell yields are usually rather small [14, 15], and cell harvesting in AMI is associated with important challenges of patient safety and logistics. On the other hand, it is likely that in AMI the optimal window for transcoronary-applied cellular therapies may be limited to the first ≈ 1–2 weeks [14], restricting the logistics of any autologous cell expansion strategies [16]. For these reasons, an intensive search is currently ongoing for clinically safe strategies using high regenerative potential allogenic cells available readily, on

an *ad-hoc* basis, and in sufficient numbers to address the clinical need to meaningfully reverse the myocardial injury burden that remains after successful AMI reperfusion in order to reduce the scar formation and adverse myocardial remodeling, preventing arrhythmic death and heart failure.

Wharton's jelly mesenchymal stem cells properties in relation to other regenerative cell types in cardiovascular medicine

Clinical translational research in the setting of AMI and heart failure to date has primarily employed autologous and non-expanded bone marrow cells [1, 26]. More recent approaches include lineage-engineered cells (applicable in a chronic but not in the acute setting) [16]) and mesenchymal cells [18]. The use of bone marrow hematopoietic stem cells or mesenchymal stem cells from the bone marrow, adult organs and fetuses for myocardial regeneration faces the disadvantages of invasive isolation, limited cell numbers and ethical constraints [6]. Allogenic cells, on the other hand, require no patient harvest and can be available in numbers larger than in the case of autologous cells. But allogenic cells elicit, in principle, an antigen-specific immune response of rejection by the recipient [29]. This is manifested by the host generation of specific anti-donor antibodies directed against the allogenic mesenchymal stem cells that may lead to immune-mediated elimination of the allogenic cells [29, 30]. Nevertheless, allogenic mesenchymal cells have been used in the context of myocardial regeneration in animal models of AMI [18, 31] (where they were shown to home to the infarct border zone [31], similar to the bone marrow-derived hematopoietic $CD34^+$ cells in humans [15, 32]), and there is at least one recent report of human cardiac use of allogenic bone marrow-derived mesenchymal stem cells [33]. The immune responses elicited by conventional allogenic mesenchymal cells are complex [29], and no long-term or large-scale safety data are available yet.

Apart from ethics, immuno-rejection is the principal biologic limiting factor with (naturally allogenic) embryonic stem cells [6], whereas induced pluripotent stem cells face the key hurdle of potential tumorigenesis [6]. In that context, WJMSCs are unique as they are non-controversial, can be harvested painlessly in abundance, are self-renewing and highly proliferative, possess stemness properties that last several passages *in vitro*, are multipotent but do not induce tumorigenesis even though they have a number of embryonic cell markers [6, 11]. Expansion of WJMSCs is not associated with any loss of genetic stability [7], as these cells are not susceptible to spontaneous malignant transformation [34]. Importantly, WJMSCs do not express major histocompatibility complex class II (HLA-DR) antigens or co-stimulatory immune response-associated surface antigens CD40, CD80, and CD86 [35]. When placed in an allogenic environment, WJMSCs are unable to elicit the response of immune-mediated rejection or proliferation of host immune cells [5, 30, 35]. Thus in contrast to other allogenic mesenchymal cell types [30], WJMSCs do not elicit an allogenic immune response of transplant rejection [5, 30, 35].

In short, WJMSCs are uniquely immune-privileged; they are not rejected by an allogenic host and thus their allogenic transplantation is associated with no need for immunosuppression [5, 30, 35]. In addition, WJMSCs can be safely expanded to "as needed" quantities [5, 7, 10], can be phenotypically selected on a large scale to create repositories of distinct WJMSC populations [36], and – if needed – can be further reprogrammed/engineered towards the desired cell lines [10, 13, 37]. Moreover, evidence is accumulating for WJMSCs' successful use in engineering biological cardiac tissues [38–40] and an allogenic non-rejectable bioartificial heart [41].

Evidence for functional regenerative effect of WJMSCs in pre-clinical studies in AMI

The unique combination of high angiogenic and cardiogenic potential of WJMSCs at bench [7, 11] with their low immunogenicity [35] makes these cells an important candidate for systematic *in-vivo* evaluation of myocardial regenerative capacity [4]. Indeed, recent evidence in a porcine model of AMI demonstrated functionally significant myocardial regeneration capacity of WJMSCs [9]. In particular, Zhang *et al.* [9] found that 40×10^6 WJMSCs transplanted by direct injections into the acutely infarcted area in the pig not only stimulated new vessel formation but also survived and differentiated into cardiomyocytes and endothelial cells and promoted recruitment and differentiation of native cardiac stem cells. In addition, WJMSC transplantation reduced apoptosis and fibrosis in the infarcted myocardium. A non-treated control group and a control group treated with placebo (phosphate-buffered saline) injections (sham procedure) were used as comparators. Overall, the multi-factorial regenerative effect of 40×10^6 WJMSCs on the infracted tissue *in situ* translated into enhancement of viable myocardium significant both statistically and functionally [9].

Taken together, evidence in large-animal AMI models shows, consistent with tissue data *ex vivo* [2, 11, 12] and small-animal AMI data [8, 10, 13], a functionally significant effect of reduction of post-infarct adverse remodeling and improvement of left ventricular function.

Choice of WJMSC dose for the human AMI study with transcoronary cell delivery

Evidence in experimental models of AMI shows that the therapeutic effect of progenitor cells is, in principle, dose-dependent [27]. Clinical evidence is overall consistent with the animal work, as it indicates a positive correlation between the cell dose and the effect on LVEF

measured by echocardiography [28] or magnetic resonance imaging [26], suggesting that maximized cell doses should be used in the clinical setting. Nevertheless, there is also evidence that transcoronary delivery of (too) large cell doses (such as in the order of ≈ 50–75 million) can become harmful to myocardium via impairment of coronary blood flow in the IRA territory and producing microinfarcts that occur most likely via distal embolization and microvascular plugging [20, 28, 42, 43].

Not only the absolute cell dose but also the cell infusion rate may be associated with impairment of IRA flow and myocardial injury demonstrated by an increase in troponin levels and new microinfarcts on histopathology. In the sheep, an adverse effect of mesenchymal precursor cell trans-IRA infusion on epicardial flow associated with an increase in troponin levels and microinfarcts on histopathology occurred with doses exceeding 40 million cells and infusion rates ≥ 2.5 million cells per minute, while lower cell doses and infusion rates had no effect on epicardial perfusion or troponin release/microinfarct formation [20]. While a maximal total cell dose of 37.5 million was found to cause no acute myocardial injury in the sheep model of acute myocardial infarction in one study [20], a pilot study by another group showed, in a similar model, no late gadolinium enhancement on cardiac magnetic resonance imaging and only a small rise in troponin with the mesenchymal cell dose of 25 million (which might be due to the use of a coronary-occlusive rather than non-occlusive cell delivery system) but a substantial troponin rise and a substantial *de novo* late gadolinium enhancement occurred only with the dose of 75 million cells [42]. For these reasons, in the present human study, an additional 20% safety margin was applied against the apparent myocardial safety of the 37.5 million mesenchymal cell dose in the study by Houtgraaf *et al.* [20]. This resulted in our selection of the dose of 30 million WJMSCs and infusion rate of 2 million cells per minute for the human study protocol. Our results demonstrated that trans-IRA infusion of 30 million WJMSCs at the rate of 2 million cells per minute caused no deterioration of IRA epicardial flow (routine TIMI flow grade assessment) or tissue flow (cTFC evaluation). Moreover, no study subject showed a troponin increase ≤ 24 h after transcoronary cell transplantation.

Thus our findings are consistent with lack of any deleterious myocardial effect of WJMSC administration according to the present study protocol.

Safety aspects of WJMSC therapy

There are at least three aspects of the safety of WJMSC therapy: (1) biological safety of cell harvest, banking, *ex-vivo* expansion and preparation for human use; (2) procedural safety of WJMSC transcoronary transfer that needs to include minimized risk of myocardial necrosis through microvessel plugging; and (3) short-and long-term clinical safety of the allogenic biologic material use in humans.

The present study identified transient temperature rise in a temporal relation to WJMSC transcoronary transfer in one subject as the only adverse effect potentially associated with WJMSCs.

An association between WJMSC transfer and transient fever would be unsurprising, as this has been established in the mesenchymal stem cell clinical use literature [44]. Although no specific data in relation to WJMSCs seem to be available, this potential side effect has been reported using both autologous and allogenic mesenchymal cells, like in various human studies, and the overall odds ratio for transient fever was found to be at least 5-fold greater with mesenchymal cells than with placebo [44].

The temperature rise in a WJMSC-treated patient in our study was only transient, and it was fully responsive to paracetamol, leading to resolution without any sequel. As fever can occur in recent acute myocardial infarction in the absence of any cause other than myocardial necrosis (and the likelihood of its occurrence increases with the infarct size) [24], further work in a large patient cohort including WJMSC-exposed and non-exposed subjects is needed to establish the prevalence of transient body temperature rise in potential relation to transcoronary WJMSC transfer. Indeed, further clinical studies in larger patient samples will need to continue close monitoring for any potential clinically relevant side effects of cardiovascular regeneration therapies using WJMSCs.

Limitations

The modest number of study participants may be considered a relative limitation of our study. Nevertheless, the present work meets the criteria of a first-in-man feasibility and safety study, while we believe that further patients should be evaluated already within the framework of a randomized controlled study with blinded analysis of potential therapeutic effects. Also, in the present study we did not perform any safety-focused WJMSC dose-response analysis. Apart from the requirement of large patient numbers for any dose-safety analysis, such potential evaluation was considered ethically unacceptable in the human setting. Rather, our choice of the WJMSC dose was based on careful analysis of the preclinical data (see above), and our selection involved the peak safe dose in large mammals with an additional 20% safety margin.

All in all, the lack of any negative effect of WJMSCs in the dose used in the present study on epicardial flow or myocardial tissue perfusion, and no troponin release in relation to cell transfer, are consistent with myocardial safety of the trans-IRA administration of 30 million WJMSCs as per the present human study protocol.

Conclusions and further directions

This study demonstrated the feasibility and procedural safety of WJMSC use as off-the-shelf cellular therapy in human AMI, and it suggested WJMSC cardiac transfer clinical safety throughout the follow-up period.

The present findings justify further investigation of the therapeutic potential of WJMSCs in humans with large AMI within the framework of a randomized, placebo-controlled clinical study appropriately powered for pre-specified therapeutic endpoints. Consistent with the concept that regenerative cell effective uptake in the infarct area is an obligatory pre-requisite for seeking any therapeutic effect [15], further work needs to establish, as a bridge to the clinical outcome study, the magnitude of myocardial retention of WJMSCs in the therapeutic target zone in relation to that of other cell types used thus far in major clinical investigations [15, 17, 45].

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Conflict of interest

The authors declare no conflict of interest.

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