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ORIGINAL ARTICLE

Allogeneic umbilical cord mesenchymal stem cell conditioned medium (secretome) for treating posterior cruciate ligament rupture: a prospective single-arm study

Sholahuddin Rhatomy[1,2] · **Jeanne Adiwinata Pawitan**[3,4,5] · **Trie Kurniawati**[6] · **Jessica Fiolin**[7] · **Ismail Hadisoebroto Dilogo**[4,8,9]

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Abstract

Background Outcomes of the current management of posterior cruciate ligament (PCL) rupture are still unsatisfactory. Recent literature demonstrated the efficacy of the paracrine action of mesenchymal stem cells (MSC) in ligament rupture healing. This study aimed to evaluate the outcome of arthroscopic administration of allogeneic umbilical cord-derived MSC (UC-MSC) conditioned medium (secretome) for the treatment of PCL rupture.

Patients and methods This is a prospective study including 12 individuals with PCL rupture grade 1 or 2 who were performed arthroscopy and secretome administrations. The functional and radiologic outcome of the knee was examined one year following intervention.

Results Preoperatively, posterior drawer test revealed three cases of grade 2+ and nine cases of grade 1+, whereas the final follow-up revealed two cases of grade 2+ and ten cases of grade 1+ PCL rupture. At final follow-up, the mean scores for the IKDC, modified Cincinnati, and Lysholm were 90.58 ± 4.30, 90.90 ± 2.15, and 89.42 ± 3.16, respectively. The means of the serial hop tests were 90.33, 94.16, 93.66, and 95.33 for single, triple, crossover, and time hop tests, respectively. Five patients were able to resume competitive sport after an average of 25.8 weeks (25–38). The final MRI analysis revealed that six knees (50%) regained PCL continuity with low signal intensity, five knees (41.6%) returned near-normal PCL continuity, and one knee (8.3%) regained PCL continuity but with deformed outlines.

Conclusions Short-term follow-up indicated that the secretome generated from allogenic UC-MSC produces excellent functional and radiographic results in grade I-II PCL rupture.

Keywords Posterior cruciate ligament (PCL) rupture · Secretome (conditioned medium) · Umbilical cord mesenchymal stem cell (UC-MSC) · Functional outcome · Radiographic outcome

Introduction

Posterior cruciate ligament (PCL) repair is a proven procedure for improving patients' quality of life and returning them to their pre-injury activities. However, posterior knee laxity and stiffness frequently persisted following surgery [1]. Nowadays, numerous studies are being conducted on ligament regeneration using stem cells, minimally invasive surgical procedures, and pain management following surgery to ensure a smooth rehabilitation process [2].

✉ Sholahuddin Rhatomy
doktergustomrhatomy@yahoo.com

Extended author information available on the last page of the article

Although stem cell-based techniques have been touted as promising in the regeneration of ligaments and tendons, these modalities are constrained by potential safety problems, such as tumorigenicity, highlighting the importance of acellular approaches. Numerous studies have demonstrated that stem cells release numerous components important in musculoskeletal regeneration, including exosomes, growth factors, and the extracellular matrix [3]. As a mixture of these components, stem cell-conditioned media (CM) demonstrates promise for tissue healing. Increasing evidence supports the use of CM for wound healing, muscle degeneration prevention, and organ function restoration [4, 5]. Until recently, however, trials testing the outcome of secretome injection for ligament injury were uncommon. On this basis, we anticipated that utilizing arthroscopy, CM injections into

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ruptured PCL could promote and enhance ligament repair and thereby improve patients' quality of life.

Materials and methods

Study design

This prospective study was conducted at Soeradji Tirtonegoro General Hospital in Klaten, Central Java, Indonesia, between October 1, 2019, and October 30, 2020. The study was authorized by the Medical and Health Research Ethics Committee of Faculty of Medicine, Public Health and Nursing, Universitas Gadjah Mada (KE-1113/EC/2019). Consent was acquired in writing from subjects prior to their participation. Patients were included if they had ruptured their PCL without rupturing another ligament and had failed non-operative therapy. Patients having any other ligament rupture, meniscal damage, chondral lesion, lower limb deformity, or accompanying lower extremity fracture were excluded from the study. Clinical examination (posterior sag sign, posterior drawer test), radiologic knee Telos stress image, and 1.5 Tesla magnetic resonance imaging (MRI) were used to identify PCL rupture. Each individual was educated about their knee issue and the techniques that would be used to treat it.

Allogeneic umbilical cord mesenchymal stem cell conditioned medium (UC-MSC Secretome) preparation

The secretome was isolated from the cultured mesenchymal stem cells from the umbilical cord's fifth passage. To preserve the growth factor components, the frozen secretome was progressively thawed by incubation at 4 °C for 16 h. Next, the secretome was transferred to a 50 ml tube and centrifuged for 10 min at 2000*g* to remove debris. Using a 0.45 m porous filter, a portion of the supernatant was filtered to remove smaller particles. Then, the filtrate underwent continued filtering using a 0.22 m porous sieve and packing in plastic polypropylene packaging that is resistant to temperatures of up to 80 °C. Sterility and potential in the form of total protein content and potential growth factors were checked as part of the quality assurance process for the end products [6, 7].

Surgical technique

All arthroscopic surgeries were conducted by a single orthopedic physician (S.R.). Patients were placed in supine position. In the proximal thigh, a tourniquet was applied, and then, typical anterolateral, anteromedial, and auxiliary portals were employed. Through typical anteromedial and lateral portals, a regular arthroscopy evaluation was performed. Allogeneic UC-MSC secretome 10 cc was injected into the remains of the PCL (Fig. 1).

Post-operative rehabilitation

For two weeks, the afflicted knee was braced in extension. After two weeks, quadriceps strengthening activities were begun, followed by range of motion exercises until 90° flexion was attained. This program was then gradually raised until complete flexion was obtained after eight weeks. After two weeks, partial weight bearing was permitted, and full weight bearing was permitted after four weeks. After five months, patients were able to resume normal daily activities. Noncontact sports were permitted after six months. Patients were evaluated using a hop test and then allowed to participate in competitive sports after 12 months. The author and a professional physiatrist oversaw all post-operative rehabilitation.

Outcome measures

The following outcomes were evaluated in this study: (1) clinical knee stability as determined by the posterior drawer test; (2) functional knee score as determined by the International Knee Documentation Committee (IKDC), modified Cincinnati, and Lysholm Knee Scale; (3) hop testing in serial (single hop, triple hop, crossover hop, and time-based hop tests); and (4) MRI examination.

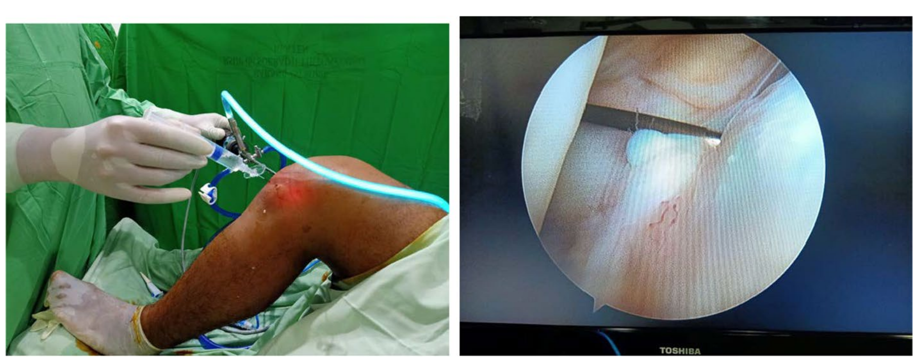

Fig. 1 Arthroscopic administration of secretome UC-MSC

To minimize bias, the MRI examinations were performed on all patients using the same parameters. Sagittal and coronal T2-weighted and proton density-weighted (PDW) fast spin echo images, as well as axial fat-saturated PDW fast spin echo images, were acquired using a 1.5-T magnetic resonance imager (Signa; GE Medical Systems, Milwaukee, WI) equipped with a quadrature knee coil. Knee imaging parameters included a repetition time/echo time ratio of 2000/20 for PDW images and 2000/80 for T2-weighted images, an echo train length of 8–12, a 3-mm slice thickness, a 1-mm interval, a 256 192 matrix, a scan time of 2 min 34 s to 3 min 28 s, and a 14-cm field of view.

Consensus evaluation of the MRI studies was conducted by one experienced musculoskeletal radiologist who was blinded to the clinical history and initial MRI interpretations. The status of injured PCL on MRI was determined using a separate scale for initial and follow-up MRI tests due to their various configurations and time periods following injury. Initial MRI scans were graded using the grading scale proposed by Grover et al. [8] Grade 0 has a continuous, low-intensity signal that corresponds to a normal PCL; grade I has areas of increased signal within the ligament but with intact borders, indicating an intrasubstance injury; grade II has areas of increased signal with one border intact, indicating a partial tear; and grade III has areas of increased signal with one border intact (Fig. 2). PCL status was classified as almost normal, continuous but deformed or thinned, and discontinuous with increased signal intensity on a follow-up MRI scan (Fig. 3).

A total of 16 continuous PCL pictures were classified as those with a low signal intensity that represented the PCL that extended from the femur to the tibia, regardless of shape or configuration.

Fig. 2 Pre-operative T1 sagittal image of grade 1–2 PCL rupture showing slight disruption of PCL continuity

Fig. 3 Post-operative T1 sagittal MRI showing normal PCL increase in continuity

Statistical analysis

Demographic data were analyzed using descriptive statistics. To compare the clinical functional knee and ankle scores, repeated measures t-tests were used. SPSS 26.0 was used for all analyses (IBM SPSS, Atlanta, GA). $P < 0.05$ was considered significant.

Results

Twelve patients met the criteria for inclusion. The mean body mass index (BMI) was within the normal range. A total of 83% of patients (10 knees) underwent right knee surgery (Table 1).

Clinical assessment

The posterior drawer test was performed without anesthesia at an outpatient facility. Preoperatively, there were three cases (25%) of grade 2+, nine cases (75%) of grade 1+, and no cases of grade 3 or 0. At the conclusion of the study, there were two cases (16.6%) of grade 2+ and ten cases (83.4%) of grade 1+ (Table 2).

Functional knee score

At final follow-up, the mean IKDC, modified Cincinnati, and Lysholm scores were 90.58 4.30, 90.90 2.15, and 89.42

Table 1 Demographic data

Age (mean ± SD, range)	28.5 ± 6.44 (19–58)
Gender	Male: 8; Female:4
Body Mass Index (mean ± SD, range)	22.07 ± 3.0 (22–34)
Side of knee	Right: 10; Left: 2

SD, standard deviation

Table 2 Distribution of posterior drawer test scores at pre-operative state and final follow-up

Score	0	+1	+2	+3
Preoperative	0	9	3	0
Final follow-up	0	10	2	0

3.16, respectively. When compared to preoperative functional scores, these outcomes were remarkably significant (Table 3).

Serial hop tests

The means of the serial hop tests for single, triple, crossover, and time hop tests were 90.33, 94.16, 93.66, and 95.33, respectively. The data are depicted in Table 4.

Return to sport

Five patients (41.7%) were able to return to competitive sports on the same level as before the injury (25 to 38). By 32.75 weeks, four patients (33.3%) could resume non-competitive sports (24 to 28). Three patients (25%) were unable to participate in sports and were limited to everyday activities until the last follow-up (1 year after surgery) (see Table 5).

Magnetic resonance imaging

Initial MRI examinations classified nine knees as grade I and three as grade II (Fig. 2). A follow-up MRI investigation revealed that six knees (50%) restored PCL continuity with modest signal intensity: 5 knees (41.6%) demonstrated virtually normal PCL continuity, 1 knee (8.3%) demonstrated continuity but distorted contours, and none demonstrated discontinuity.

Table 4 Serial hop test at final follow-up

Type	Mean ± SD, range (%)
Single hop	90.33 ± 2.38 (88–96)
Triple hop	94.16 ± 2.32 (90–98)
Cross over hop	93.66 ± 2.38 (90–96)
Timed hop	95.33 ± 3.72 (88–98)

SD, standard deviation

Table 5 Return to sport level

Type	Final follow-up
Full competitive sport	5 (41.7%)
Non-competitive sport	4 (33.3%)
Daily activity living	3 (25%)

Complications

One patient had a minor fever and swollen knee a week after surgery; leucocyte blood count, erythrocyte sedimentation rate, and C reactive protein were all within normal limits; knee aspiration culture and sensitivity assays revealed no bacteria; and the problems improved with paracetamol.

Discussion

Numerous studies have demonstrated the efficacy of stem cells in the treatment of ligament injuries [9]. However, the use of stem cells has significant limitations, including cost, danger of malignancy, and inability to be performed in hospitals located distant from stem cell collecting sites. Numerous research on secreted factors produced from stem cells demonstrated that the released factors alone, without the stem cell, may promote tissue repair in a variety of diseases including tissue/organ injury. The secreted factors are referred to as secretome, microvesicles, or exosomes and are

Table 3 Results of Knee functional score at Final Follow up

	Mean ± SD	95% CI	*p* value
IKDC			
Pre-operative	54.98 ± 4.4 (46–60.9)	(−41.2) – (−36.30)	< 0.001
Final follow-up	93.78 ± 3.45 (86.2–100)		
Modified Cincinnati			
Pre-operative	62.66 ± 8.83 (53–86)	(−33.62) – (−24.53)	< 0.001
Final follow-up	91.75 ± 3.3 (88–100)		
Lysholm			
Pre-operative	65.41 ± 8.49 (54–80)	(−28.36) – (−18.63)	< 0.001
Final follow-up	88.91 ± 2.81 (85–94)		

CI, confidence interval; IKDC, International Knee Documentation Committee; SD, standard deviation found in the medium used to culture the stem cells; thus, the medium is referred to as conditioned medium (CM). This CM contains a variety of growth factors and tissue regenerative agents secreted by the stem cells. Numerous proteomic studies have also demonstrated that stem cells release various growth factors, revealing the presence of various growth factors and other cytokines in the CM [4, 5].

As a component of CM and as a free cell therapy, secretome is more adaptable, less expensive and can be employed as a therapeutic option in hospitals that are remote or inaccessible to stem cell harvesting laboratories. It also helps prevent future malignancy issues. Until now, no clinical trial utilizing CM for a specific disease has been reported, with the exception of two pilot studies utilizing adipose-derived mesenchymal stem cell CM for hair follicle regeneration and fractional carbon dioxide resurfacing wound healing in humans, which demonstrated favorable results. The use of CM in therapy is extremely encouraging and is likely to explode in the near future, as research on the use of CM for various diseases continues to accumulate [4, 5].

The therapy of a ruptured PCL is still ambiguous; while some research indicates that non-operative treatment is optimum, others indicate the opposite [10–16]. Several studies indicated that while PCL restoration had ideal results, it also had some post-reconstruction complications that resulted in a reduction in patients' quality of life [17, 18].

The purpose of this study was to stimulate ligament regeneration using minimally invasive techniques, free cell treatment, painless post-operative rehabilitation, and accelerated post-operative rehabilitation programs in order to avoid post-reconstruction sequelae.

Our investigation found that only one patient (8.34%) had improved knee stability following final follow-up. The majority of cases (91.64%) showed no difference from before treatment. Our study, like others, found that PCL reconstructions retain some flexibility following surgery [1, 19–21].

Functional knee scores were significantly improved compared to preoperative values using the IKDC, modified Cincinnati, and Lysholm scores. Our findings are consistent with those of previous studies [10, 13–16] on the functional outcome of solitary PCL rupture treated nonoperatively or with PCL reconstruction [11, 17, 18].

Additionally, this study demonstrated that at the final follow-up, all participants scored above 90 on the serial hop test, and five patients (41.7%) were able to return to competitive sports in the same capacity as before the injury within 25.8 weeks (25 to 38). By 32.75 weeks, four patients (33.3%) may resume non-competitive sports (24 to 28). Three patients (25%) were unable to participate in sports and were limited to everyday activities until the last follow-up (1 year after surgery). Although there are limited studies on the treatment of PCL rupture in terms of return to competitive activity, research conducted by Agolley et al. indicates that the majority of patients can return to sports following non-operative treatment [14]. The process of returning to sports is influenced by a variety of factors, including injury care, diet, rehabilitation programs, and patient psychology [14, 17, 22].

Our study's MRI findings indicated that there was some regeneration in the PCL remnant. This finding is consistent with numerous studies indicating that the PCL regenerates even when it is not repaired, because the PCL is physically covered by a synovium that is densely vascularized [12, 15]. By providing a growth factor-rich conditioned medium/secretome, it is possible to stimulate and expedite regeneration [4, 5].

Our analysis identified one patient with a moderate fever, swollen knees, and no infection parameters who responded to paracetamol oral medication. We suspect an inflammatory process as a result of the secretome injection.

Conclusions

Short-term follow-up indicated that the secretome generated from allogenic UC-MSC produces good functional and radiographic results in grade I-II PCL rupture. However, longer-term follow-up in controlled research is necessary to assess the secretome MSC's efficacy in treating grade I-II PCL rupture.

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Declarations

Ethical approval This study had been reviewed by our institutional review board and received ethical clearance number KE-1113/EC/2019.

Conflict of interest The authors declare that they have no competing interests.

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Authors and Affiliations

Sholahuddin Rhatomy[1,2] · Jeanne Adiwinata Pawitan[3,4,5] · Trie Kurniawati[6] · Jessica Fiolin[7] · Ismail Hadisoebroto Dilogo[4,8,9]

Jeanne Adiwinata Pawitan
jeanneadiwp@gmail.com

Trie Kurniawati
trie3k@ui.ac.id

Jessica Fiolin
jessica_fiolin@yahoo.co.uk

Ismail Hadisoebroto Dilogo
ismailortho@gmail.com

[1] Sport and Adult Reconstruction Division, Department of Orthopaedic and Traumatology, Soeradji Tirtonegoro Hospital, Klaten, Indonesia

[2] Faculty of Medicine, Public Health, and Nursing, Universitas Gadjah Mada, Yogyakarta, Indonesia

[3] Department of Histology, Faculty of Medicine, Universitas Indonesia, Jakarta, Indonesia

[4] Stem Cell Medical Technology Integrated Service Unit, Cipto Mangunkusumo Central Hospital, Faculty of Medicine, Universitas Indonesia, Jakarta, Indonesia

[5] Stem Cell and Tissue Engineering Research Cluster Indonesian Medical Education and Research Institute (IMERI), Universitas Indonesia, Jl. Salemba Raya No. 5, Jakarta, Indonesia

[6] Stem Cell Medical Technology Integrated Service Unit, Cipto Mangunkusumo Central Hospital, Faculty of Medicine, Universitas Indonesia, Jl. Diponegoro no. 5, Jakarta, Indonesia

[7] Orthopaedic Surgeon of Jakarta Knee, Shoulder and Orthopaedic Sport Clinic, Pondok Indah General Hospital, Jl. Metro Duta Kav UE, Jakarta Selatan 12310, Indonesia

[8] Orthopaedic and Traumatology Department, Faculty of Medicine, RS DR Cipto Mangunkusumo, Jakarta, Indonesia

[9] Stem Cell and Tissue Engineering Research Cluster Indonesian Medical Education and Research Institute (IMERI), Universitas Indonesia, Jl. Diponegoro no 71, Jakarta, Indonesia