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Autologous cultured adipose derived mesenchymal stem cells combined with hyaluronic acid hydrogel in the treatment of discogenic low back pain: a study protocol for a phase II randomised controlled trial

Jing Zhang,[1] Tianze Sun,[1] Wentao Zhang,[1] Ming Yang,[1] Zhonghai Li [1,2]

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[1]Department of Orthopedics, First Affiliated Hospital of Dalian Medical University, Dalian, Dalian, China
[2]Key Laboratory of Molecular Mechanism for Repair and Remodeling of Orthopedic Diseases, Liaoning Province, China

Correspondence to
Professor Zhonghai Li;
lizhonghaispine@126.com

ABSTRACT

Introduction Discogenic low back pain (DLBP) is a common disease, and its occurrence is closely related to intervertebral disc (IVD) degeneration. At present, none of the traditional treatment methods can repair the degenerated IVD. The emergence of stem cell therapy makes it possible to repair and regenerate IVD tissue, among which adipose derived mesenchymal stem cells (ADMSCs) transplantation therapy has become a hot spot of current research. Therefore, this trial aimed to investigate the safety and efficacy of using autologous cultured ADMSCs combined with hyaluronic acid (HA) hydrogel in the treatment of DLBP.

Methods and analysis This study is a randomised, dose-escalation, placebo-controlled, double-blind, single-centre, phase II clinical trial to evaluate the efficacy and safety of autologous cultured ADMSCs combined with HA hydrogel in the treatment of patients with DLBP. The 100 eligible patients will be randomly divided into three experimental groups with different doses and one placebo control group in a ratio of 1:1:1:1. All patients will undergo liposuction to obtain ADMSCs, followed by autologous cultured ADMSC mixtures or placebo transplantation after 3 weeks. The patients will be followed up to 24 months after the transplant. The primary end point of this trial is the Visual Analogue Scale. Secondary end points include the Oswestry Disability Index, Japanese Orthopaedic Association Scores, the Mos 36-item short form, the Modic classification, Pfirrmann grade, height and segment range of motion of the IVD, vital signs (temperature, pulse, respiration, blood pressure), blood routine, liver and kidney function, immunological examination, urinalysis and treatment emergent adverse events.

Ethics and dissemination The study protocol has been approved by the Ethics Committee of the First Affiliated Hospital of Dalian Medical University and registered in the Chinese Clinical Trial Registry. Dissemination of the results will be presented at a conference and in peer-reviewed publications.

Trial registration number ChiCTR2200058291.

STRENGTHS AND LIMITATIONS OF THIS STUDY

⇒ This study is a randomised, placebo-controlled, double-blind trial, so bias will be minimised.
⇒ The subjects of this trial are recruited from only one research centre, and the sample size is not large enough.
⇒ The subjects of this trial are patients with discogenic low back pain with single disc segment degeneration, which would limit applicability to the general population.

INTRODUCTION

Low back pain (LBP) is a common health concern worldwide. According to some studies, the point prevalence of LBP is 11.9%, and the 1 month prevalence is 23.3%. LBP is the main reason for years lived with disability and places a heavy burden on patients and society.[1–3] Discogenic LBP (DLBP) is a common source of LBP, with an overall prevalence of 26%~42%, and in the younger population, this rate reaches >80%.[4 5] DLBP gradually evolves from internal intervertebral disc (IVD) diseases such as inflammation, deformation and annulus fibrosus (AF) injury, and its key pathological process is IVD degeneration (IDD).[6 7] The clinical manifestations of patients with DLBP are recurrent LBP, especially when sitting for a long time, bending over or coughing, but there is often no positive feature of nerve root damage during physical examination. Although the pathological mechanism of DLBP has not been fully understood, it is widely believed that the sensory nerve fibres from the outer layer of the IVD grow into the interior through the fissure, and the production of proinflammatory cytokines in the degenerated IVD increases, resulting in back pain in patients.[8–10] In addition, abnormal lumbar disc activity due to long-term mechanical injury may

accelerate the progression of DLBP.[11 12] The diagnosis of DLBP requires a combination of imaging findings and long-term recurrent LBP. Clinically, MRI is an essential tool for evaluating IVD pathology, and its signal characteristics reflect the degree of IVD ageing or degeneration.[7] Many patients with DLBP are found to have decreased signal intensity in the IVD on MRI T2-weighted imaging, which is the so-called 'black' IVD, and the sagittal view shows that the posterior border of the IVD has a small, round, limited area of high signal intensity.[13 14] However, the relationship between the degree of disc degeneration on imaging and the severity of pain remains unclear, so it is necessary to rely on CT-guided discography for further diagnosis. Discography is currently the only 'gold standard' test for diagnosing DLBP. Following injection of the contrast agent into the disc, patients will experience similar pain responses, but the adjacent disc will not.[14] Based on discography, DLBP may be categorised as AF ruptured LBP and cartilage endplates (CEPs) ruptured LBP, and this classification method has clinical and theoretical support.[15] Due to the varying severity of clinical manifestations in patients with DBLP, step-by-step therapy is often used in the treatment. Traditional treatment methods include conservative, interventional and surgical treatments.[15] Most patients tend to opt for conservative treatments due to mild symptoms and a short course of the disease.These conservative treatments mainly include bed-rest, physiotherapy with microwave or infrared, oral painkillers, and functional exercises. Interventional treatments, such as epidural injections and percutaneous intradiscal therapies, are generally performed if conservative treatments fail.[16–18] With severe symptoms or ineffective conservative and interventional treatments, surgical treatments are often recommended. The most commonly used surgical procedures are interbody fusion and artificial disc replacement.[18] However, conservative treatments have limited efficacy, and interventional treatments for pain relief should be further evaluated. Although surgical treatments can effectively relieve pain, they may cause complications such as infection, nerve damage, large blood vessel damage and adjacent segment degeneration due to improper operation or care, which will further damage the body of patients.[19 20] Additionally, the best way to treat DLBP would be to slow down or even reverse the process of IDD, but neither conservative treatments, interventional treatments nor surgical treatments can do anything about it. Traditional treatments are in a dilemma, and a new treatment is urgently needed to induce repair of degenerated disc tissue.

The IVD is composed of an outer AF, a nucleus pulposus (NP) in the middle, and CEPs at the upper and lower ends. NP is the main structure of IVD, which is mainly composed of NP cells and extracellular matrix (ECM). NP cells are cartilage-like cells. The main components of ECM are COL II, proteoglycans and other matrix proteins.[21] IDD mainly occurs in NP. With the degradation of ECM and the loss of proteoglycans, these changes will reduce the structural integrity of IVD and eventually damage its function.[22] The recent deepening of the understanding of IDD has promoted the research of biological therapy, among which stem cell therapy stands out and becomes the current research hot spot. In the past few decades, human stem cell therapy indications have involved many different fields, including neurological diseases, cardiovascular diseases, diabetes, blood diseases and cancer, with exciting results.[23–26] Given the broad application prospects of stem cell therapy in regenerative medicine, people have begun to explore the application in spinal degenerative diseases. DLBP is a common spinal degenerative disease, and the key to its treatment is to repair the degenerated disc tissue and reduce the discomfort of patients. The transplanted stem cells are capable of self-replication, renewal and multidirectional differentiation, which can differentiate into chondrocytes to replace the lost NP cells, as well as promote the formation of ECM by co-culturing with NP cells.[27–29] In addition, stem cells have immunomodulatory effects and can secrete various cytokines to improve the microenvironment inside the IVD, promote the repair of degenerated IVD tissue and relieve the pain of patients.[27–29] Stem cells for the treatment of IDD come from a wide range of sources, including bone marrow mesenchymal stem cells (BMSCs) and adipose derived MSCs (ADMSCs), and a few stem cell types derived from human umbilical cord MSCs, pluripotent stem cells, NP-derived stem cells and other sources.[29 30] Among the many types of stem cells, BMSCs are currently the most studied, and their safety and efficacy in the treatment of IDD have been verified in clinical trials, bringing the dawn of stem cell clinical treatment for IDD.[31 32] However, due to the cumbersome and invasive process of obtaining BMSCs, its clinical application is limited to a certain extent.[33 34] With the deepening of research, it has been found that ADMSCs have similar chondrogenic differentiation potential compared with BMSCs. More importantly, ADMSCs have the advantages of ease of obtaining in large quantities, lower incidence of donor site and higher proliferation potential, so they may be an ideal source of stem cells for the treatment of DLBP.[33 35]

During stem cell therapy, potential complications such as leakage and osteophyte formation may occur at the infusion site, and the implanted stem cells are subject to high mechanical loads in the disc, which may reduce the viability or function of the stem cells. In order to solve these problems, various scaffolds have been designed as carriers for delivering stem cells, among which hydrogel scaffolds are more commonly used.[36–38] Among various biomaterials for the production of hydrogel scaffolds, hyaluronic acid (HA) and its derivatives have been extensively studied. HA is a naturally occurring glycosaminoglycan that is involved in vital processes such as cell proliferation, migration, angiogenesis and tissue growth. Biocompatibility, biodegradability, processability and tunable mechanical properties of HA contribute to its clinical appeal.[39–41] Therefore, the transplantation of ADMSCs combined with HA hydrogel has become a hot research topic.

The efficacy of ADMSCs in the treatment of DLBP has been verified in animal models.[42–44] In order to further verify whether stem cell therapy is also safe and effective in humans, it is necessary to conduct clinical trials. Currently, six clinical trials of ADMSCs in the treatment of IDD are registered on the ClinicalTrials.gov website, and four of them (NCT01643681, NCT03461458, NCT05011474, NCT02529566) have not published their results for various reasons. One of the remaining two clinical trials (NCT02097862) evaluated the safety and efficacy of intradiscal injection of stromal vascular fraction (SVF) in combination with platelet rich plasma in patients with degenerative disc disease.[45] There are ADMSCs and growth factors in the SVF, but the adipocyte population has been depleted. Another phase I clinical trial (NCT02338271) demonstrated the safety and tolerability of ADMSCs combined with HA hydrogel therapy.[46]

Safety is an important consideration in conducting clinical trials. Since the IVD contains cartilage tissue, researchers also pay attention to the progress made by ADMSCs in treating articular cartilage injuries. The safety of ADMSCs in repairing articular cartilage has been demonstrated in clinical trials conducted in the USA,[47 48] China,[49 50] South Korea,[51] Italy,[52] France[53] and Australia.[54] The progress made by ADMSCs in the treatment of cartilage injury has significantly increased our confidence in their application to the treatment of DLBP.

Although the data from these clinical trials preliminarily demonstrated the safety of autologous cultured ADMSCs in the treatment of DLBP, there are many shortcomings in these clinical trials, such as the limited number of samples, the lack of appropriate controls and the lack of blinding. In order to further clarify the efficacy and safety of autologous cultured ADMSCs combined with HA hydrogel therapy, a large-sample phase II trial with matched controls is required. Based on previous promising findings, we design a double-blind, randomised-controlled phase II clinical trial to evaluate the efficacy and safety of percutaneous intradiscal injection of autologous cultured ADMSCs combined with HA hydrogel scaffold in patients with DLBP.

METHODS AND ANALYSIS

Study design

This study is a randomised, dose-escalation, placebo-controlled, double-blind, single-centre, phase II clinical trial to evaluate the efficacy and safety of autologous cultured ADMSCs combined with HA hydrogel in the treatment of patients with DLBP. This trial will be conducted at the First Affiliated Hospital of Dalian Medical University in Dalian, Liaoning Province, China, and is expected to enrol 100 patients. After informed consent, patients will undergo relevant examinations, and only eligible patients can participate in clinical trials. All eligible subjects will undergo liposuction to obtain autologous cultured ADMSCs, and then the ADMSCs and HA hydrogels will be formulated into different doses of stem cell mixtures. In the third week after liposuction, subjects will receive either different doses of stem cell mixtures or a placebo transplant, followed by a 24-month follow-up. The trial was registered in the Chinese Clinical Trials Registry (http://www.chictr.org.cn) on 4 April 2022 (registration number ChiCTR2200058291). The detailed trial flow is described in figure 1.

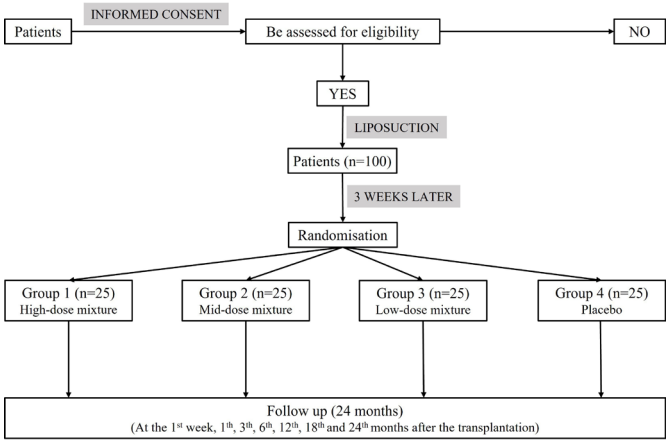

Figure 1 Study flow diagram.

Study objectives

There are two specific objectives of this phase II clinical trial:

1. To evaluate the efficacy and safety of percutaneous intradiscal injections of ADMSCs combined with HA hydrogel in the treatment of patients with DLBP.
2. To determine the optimal therapeutic dose of ADMSCs.

Simple size and calculation

As a previous study indicated that after transplantation of 18 million allogeneic BMSCs, the mean Visual Analogue Scale (VAS) Score of patients with IDD was reduced to 37.63±10.27 (10.3 reduction) after 24 months,[55] we set this indicator at 10.6 reduction after 24 months of transplantation treatment. Furthermore, we assumed that α was 0.05, β was 0.1 and the dropout rate was 20%. Therefore, the calculated sample size for each group was 25, and the total sample size was 100 patients.

Eligibility criteria

Patients with LBP who obtained informed consent will only be allowed to participate in this clinical trial if they meet all the inclusion criteria and none of the exclusion criteria. The inclusion criteria are shown in box 1, and the exclusion criteria are shown in box 2.

Recriument

Participants will be recruited from three sources. First, the potentially eligible hospitalised patients diagnosed with DLBP will be approached and recommended for enrolment in this study. Second, physicians will generate a list of patients with DLBP who have not undergone surgery from the hospital's electronic records. Researchers or physicians will contact these

Box 1 Inclusion criteria

1. Patients who are male or female and whose age must be 18 years or older.
2. Chronic LBP is accompanied by more than two (including two) clinical manifestations: increased pain when abdominal pressure increases such as cough and sneezing, increased pain when sedentary, forward bending or lifting heavy objects, difficult to relieve or unable to maintain the same posture, and pain relief when lying flat and resting.
3. Patients with LBP lasting 1 year or longer after conservative treatment.
4. VAS≥4.
5. ODI≥30%.
6. MRI shows that the CEPs of the lumbar IVD is Modic type I or II.
7. MRI shows that the L4-5 IVD is Pfirrmann grade 3, 4 or 5.
8. Discography of lumbar IVD(s) identified as degenerated by MRI show(s) that the patients have only one disc of L4-5 level with similar pain as usual.
9. Patients with ≥20% loss of lumbar disc height compared with normal adjacent discs based on X-ray assessment.
10. Patients with no active infection (such as HBsAg, HIV, cytomegalovirus and rubella virus).

CEPs, cartilage endplates; HBsAg, hepatitis B surface antigen; IVD, intervertebral disc; LBP, low back pain; ODI, Oswestry Disability Index; VAS, Visual Analogue Scale.

Box 2 Exclusion criteria

1. Patients with spondylitis or vertebral fractures.
2. Surgery is required for patients with severe lumbar spinal stenosis or prolapse of the lumbar NP resulting in severe nerve compression and pain in the lower limbs.
3. Patients who have received any intradiscal injection procedure (eg, injection of corticosteroids, methylene blue, dextrose, or glucosamine and chondroitin sulfate) within 1 year prior to receiving transplantation therapy.
4. Dynamic X-ray examination of the lumbar spine shows that the adjacent vertebral body slips >3 mm or is angled >15°.
5. Patients with severe osteoporosis with a BMD T value of −2.5 or lower on DEXA.
6. Pregnant or lactating women, or women who become pregnant within 24 months after receiving intervention.
7. Patients with mental illness or drug addictions or alcohol addictions or those incapable of understanding the purpose or methods of the study.
8. Patients with a history of various systemic diseases such as cancer, autoimmune disease, blood diseases, kidney diseases or liver diseases.
9. Patients who are allergic to HA, contrast agents or local anaesthetics (eg, lidocaine, bupivacaine).
10. Patients who have previously used any other cell product and/or plan to participate in any other stem cell clinical trial during the 2-year follow-up period

BMD, bone mineral density; DEXA, dual-energy X-ray absorptiometry; HA, hyaluronic acid; NP, nucleus pulposus.

patients by phone and recommend them to participate in the study. Third, physicians will post study flyers at the outpatient department and the official website for patients diagnosed with DLBP at other hospitals, and if they are interested in this study, we will initiate the screening process.

ADMSCs preparation and culture

All eligible patients have 150 mL of subcutaneous adipose tissue harvested by clinicians in the operating room through liposuction under local anaesthesia 3 weeks before transplantation, and the patients are discharged after a 4-hour observation period. The harvested adipose tissue will be shipped to the cell factory. ADMSCs will be obtained by washing, enzymatically dissociating and centrifuging the adipose tissue using strict aseptic techniques, The cells will be plated on flasks and cultured at 37°C in a humidified incubator under a 5% CO_2 atmosphere. ADMSCs used in this clinical trial will be obtained from cultured third-generation cells. These cell surface markers are positive for CD44, CD73, CD29 and negative for CD45 by flow cytometry, and the final products are tested to rule out the growth of aerobes, anaerobic bacteria and mycoplasma. In addition, cytogenetic analysis will be performed to rule out abnormal karyotypes. The cells are suspended at a concentration of 20×10^6 cells/mL of normal saline/vial, and these suspensions are transported to the operating room of the institute in a cold box at approximately 4°C.

Preparation of HA hydrogel for cell delivery

The molecular weight of HA plays an important role in modulating the inflammation in LBP during disc repair. It is generally believed that high molecular weight HA inhibits the activation of proinflammatory cytokines and matrix-degrading enzymes, whereas low molecular weight HA promotes inflammatory and tissue remodelling.[56–58] The implantable HA hydrogel in this trial is prepared by mixing 1% FCH-200 (fluorous-core nanoparticle-embedded hydrogel) with fibrin solution and incubating it at room temperature for 15 min. FCH-200 is a high molecular weight HA (molecular weight: 1800–2200 kDa), purchased from Kikkoman Bio Chemifa. A previous study has shown that this HA promotes the aggregation of ADMSCs and induce their differentiation towards cartilage.[59] Therefore, the scaffold material selected for this clinical trial is HA hydrogel with FCH-200 as the gel matrix.

Transplantation of ADMSCs in combination with HA hydrogel

Based on discographic findings and confirmation of IDD with T2-weighted MRI, symptomatic discs are selected for transplantation. Under C-arm fluoroscopy, clinicians used spinal needles to percutaneously implant different doses of ADMSCs with the HA hydrogel or saline into the IVD centre via a standard posterolateral approach. The needle diameter of the spinal needle is 22 G. After the transplantation, the

patients will be asked to restrict physical activity for 2 weeks.

Randomisation and blinding

All the selected subjects are randomly assigned into the group according to the ratio of 1:1:1:1, and the statistician uses R software to generate a random sequence; a total of four groups with 25 cases in each group. In this experiment, the random envelope method is used for grouping concealment. Subjects who meet the eligibility criteria are assigned random numbers (001–100) in the order of enrolment, and they then open the random envelopes with the corresponding numbers to obtain the corresponding grouping information.

This trial adopts a double-blind design. The injections of the control group and the injections of the experimental groups are completely identical in appearance. During the transplantation treatment, both the patients and the clinicians performing the transplantation will be blinded. A statistician generates random numbers and corresponding drug codes, and then distributes and packs the drugs according to the codes, and prepares corresponding emergency letters. The computer programme that generates the random numbers and the drug codes are kept as a blind bottom. Blind bases are in duplicate and kept in the trial responsible unit and agency, respectively. A two-level blind design was adopted; the first level was the group corresponding to each case number and the second level was the treatment corresponding to each group. The pharmacist dispenses the test drug and the placebo into a small sachet according to a single infusion dose. The sachets have the same shape and are opaque, and are marked with the serial number corresponding to the intervention category determined by the random number. Allocation tables recording serial numbers, random numbers and group markers are kept in triplicate by the trial designer, the pharmaceutical company and the pharmacy, respectively. Neither the trial designer nor the pharmacist participate in the trial.

Intervention

During the third week after liposuction, the subjects receive different doses of stem cell mixtures or placebos transplant. Subjects in the high-dose group receive a mixture that includes 1 mL of stem cell suspension (20×10^6 cells/disc), and 1 mL of HA hydrogel; subjects in the mid-dose group receive a mixture that includes 0.5 mL of stem cell suspension (10×10^6 cells/disc), 0.5 mL of normal saline and 1 mL of HA hydrogel; subjects in the low-dose group receive a mixture that includes 0.25 mL of stem cell suspension (5×10^6 cells/disc), 0.75 mL of normal saline and 1 mL of HA hydrogel; subjects in the control group receive 2 mL of normal saline injection. Because the purpose of this study is to investigate the efficacy and safety of stem cells combined with HA hydrogel in the treatment of DLBP, there is no separate HA hydrogel control group. During the transplant process, neither the subjects nor the clinicians know the specific transplant treatment drug and doses.

Table 1 Outcome measures and time frames

Outcome measures		Time frames
Primary outcome measures	VAS Score	At baseline, 1 week, 1, 3, 6, 12, 18 and 24 months
Secondary outcome measures	ODI Score	At baseline, 1 week, 1, 3, 6, 12, 18 and 24 months
	JOA Scores system	
	SF-36 health survey score	
	The Modic changes of the IVD	At baseline, 6, 12, 24 months
	The Pfirrmann grade of the IVD	
	Disc height	
	Segment range of motion	
	Vital signs: temperature, pulse, respiration, blood pressure	At 1 week, 1, 3, 6, 12, 18 and 24 months
	Blood routine	
	Liver and kidney function	
	Immunological examination	
	Urinalysis	
	Treatment emergent adverse event	

IVD, intervertebral disc; JOA, Japanese Orthopaedic Association; ODI, Oswestry Disability Index; SF-36, the Mos 36-item short form; VAS, Visual Analogue Scale.

Outcome evaluation

The primary end point of this trial is improvement in VAS from baseline (prior to the transplantation) at each follow-up time point. Secondary end points include Oswestry Disability Index, Japanese Orthopaedic Association Scores, the Mos 36-item short form, the Modic classification, Pfirrmann grade, height and segment range of motion of the IVD, vital signs (temperature, pulse, respiration, blood pressure), blood routine, liver and kidney function, immunological examination, urinalysis, and treatment emergent adverse events (AEs). Each follow-up time point will be conducted by telephone and outpatient contacts. Patients or their families will be reminded by phone the day before the follow-ups. The corresponding outcome measures and their time frames are listed in table 1.

Adverse events

AEs are defined as adverse medical events that occur after the patient signs informed consent until completion of the follow-up period. AEs include abnormal laboratory results, symptoms or diseases. If the AE is confirmed to be a treatment-related adverse reaction, it will be reported

according to the adverse reaction reporting procedure of the research centre. Once an AE occurs, the clinician will conduct necessary treatment according to the patient's condition and decide whether to suspend the clinical study. Serious AEs (SAEs) refer to life-threatening medical events such as paralysis, tumours, serious infections and even death of patients during clinical trials. In terms of SAEs, clinicians should treat it as an emergency and will follow the principle of priority treatment. The researcher will report to the head of the centre and the ethics committee of the research unit within 12 hours of the first learning, and report to the team leader within 24 hours or no later than the second working day. At the same time, researchers must handle the communication and aftermath of the subjects and their families.

Withdrawal

Discontinuation can occur as a result of SAEs such as paralysis, tumours, serious infections or even death, other serious diseases limiting participation, or withdrawal by the subject requesting the study to be stopped. Those subjects who withdraw from the trial will have their withdrawal reasons and all observations recorded. New participants will not be recruited to replace withdrawn participants.

Data and safety monitoring

The members of the Data and Safety Monitoring Board (DSMB) are independent of the trial investigators and have no competing interests. Clinical safety and efficacy data collected at the time intervals specified in the protocol will be reviewed and evaluated by the DSMB. The DSMB will be notified if the safety data threshold exceeds a predefined threshold. Additionally, the DSMB will conduct an interim analysis of all AE occurrences every 6 months during the course of the study. All investigators and monitors will have access to the electronic trial data during the data collection period; after completion of the trial, the data will also be accessible to statisticians. All of the data will be provided to the DSMB.

Data collection

The data generated during the trial will be recorded in the original medical record and the case report form (CRF). To ensure that the data are entered accurately into the CRF, quality control personnel check the consistency of the CRF data with the original record. There are eight data collection points: baseline, 1 week, 1 month, 3 months, 6 months, 12 months, 18 months and 24 months. Research records will be submitted within 3 days of the completion of the data collection to the research leader for review and all data will be submitted within 10 days to the project leader. Next, the auditor will examine each original research record to ensure that the clinical trial data records are accurate, precise and standardised. Data checks and entries will then be disposed off by the statistical data manager and analysed by the statisticians.

Patient and public involvement

The patients and public were not involved in the design, or conduct, or reporting or dissemination plans of our research.

Statistical analysis

Statistical analysis will be performed using SPSS V.22.0 software. All statistical tests are two-sided, the test level is α=0.05, and the CI is 95% confidence level. The primary focus of the data analysis is to determine the effect of any treatment at each follow-up point (1 week and 1, 3, 6, 12, 18 and 24 months post-transplant). In addition, linear mixed models are applied to assess differences in treatment effect between groups (20×10^6 cells/disc, 10×10^6 cells/disc and 5×10^6 cells/disc) at each follow-up point. According to the type of variables and data distribution, t test, analysis of variance or non-parametrical test is used for measurement data, and χ^2 test is used for enumeration data. The safety evaluation is mainly based on descriptive statistical analysis, listing AEs, SAEs, the number of times leading to AEs and calculating the incidence.

Rules for unblinding

Unblinding is carried out according to normal procedures if no subject pregnancy or other emergency occurs during the course of the trial. First, after checking the CRF and signature, the grouping of subjects will be clarified through first-level unblinding for statistical analysis. Second, after the statistical analysis, the corresponding control and experimental groups will be identified through secondary unblinding to evaluate the efficacy of stem cell injections. If there is an emergency, emergency unblinding can only be performed if the investigator must have information on the treatment assignment of subjects in emergency. The investigator will unblind according to the treatment information provided by the subject in the emergency letter, and then complete the unblinding record form and note it on the CRF. After the trial, the number, reason, scope and time of emergency unblinding should be described and analysed as a reference for the evaluation of efficacy and safety.

DISCUSSION

This phase II clinical trial will answer two key questions for patients and the scientific community. First, whether autologous cultured ADMSC combined with HA hydrogel is safe and effective in the treatment of DLBP. Second, what is the optimal dose of ADMSCs for the treatment of DLBP. Although many studies have been conducted on BMSCs, and the results of phase III clinical trials have also demonstrated their efficacy, the invasiveness of obtaining BMSCs makes many patients hesitant. The emergence of ADMSCs perfectly fills this gap and makes stem cell therapy more acceptable to patients, which will greatly promote stem cell therapy to the clinic. However, stem cell therapy also faces challenges. First of all, in vitro studies have shown that the harsh microenvironment inside the

degenerated IVD will affect the biological behaviours of stem cells, such as viability, proliferation and differentiation, and then affect the therapeutic effect of stem cell therapy.[60 61] Second, the clinical research on ADMSCs is still in a relatively immature stage, and the specific therapeutic mechanism of stem cells is still lacking in-depth understanding. Therefore, it is necessary for us to carry out more clinical trials to further explore. Although there are relatively few clinical trials for DLBP treatment, ADMSCs are still a promising type of MSCs. The successful implementation of this clinical trial will provide data support for subsequent phase III clinical trials, and will also significantly promote the clinical application of ADMSCs. DLBP patients are about to usher in a new era of ADMSCs therapy.

ETHICS AND DISSEMINATION

The study protocol has been approved by the Ethics Committee of the First Affiliated Hospital of Dalian Medical University and registered in the Chinese Clinical Trial Registry. The results will be presented at a conference and in peer-reviewed publications. An insurance company will provide insurance coverage for damages emerging from the trial.

Protocol amendments

All protocol amendments will be evaluated by the Ethics Committee and the Chinese National Medical Products Administration, following the principles of Good Clinical Practice and national legislation. All modifications of the study protocol will be communicated by updating the trial registry at the Chinese Clinical Trials Registry (http://www.chictr.org.cn).

Dissemination policy

Output from this study will include journal publications, conference presentations and community reporting. Output will not identify participants.

Contributors ZHL contributed to the conception and design of the study. JZ wrote the first draft of the manuscript. WTZ, TZS and MY supervised the manuscript. All authors contributed to manuscript revision and approved the submitted version.

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Competing interests None declared.

Patient and public involvement Patients and/or the public were not involved in the design, or conduct, or reporting, or dissemination plans of this research.

Patient consent for publication Not applicable.

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ORCID iD

Zhonghai Li http://orcid.org/0000-0003-4735-1193

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