Intervertebral Disc Repair by Allogeneic Mesenchymal Bone Marrow Cells: A Randomized Controlled Trial

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Background. Degenerative disc disease often causes severe low-back pain, a public health problem with huge economic and life quality impact. Chronic cases often require surgery, which may lead to biomechanical problems and accelerated degeneration of the adjacent segments. Autologous mesenchymal stromal cells (MSC) treatments have shown feasibility, safety and strong indications of clinical efficacy. We present here a randomized, controlled trial using allogeneic MSC, which are logistically more convenient than autologous cells. Methods. We randomized 24 patients with chronic back pain diagnosed with lumbar disk degeneration and unresponsive to conservative treatments into 2 groups. The test group received allogeneic bone marrow MSCs by intradiscal injection of $25 \times 10^6$ cells per segment under local anesthesia. The control group received a sham infiltration of paravertebral musculature with the anesthetic. Clinical outcomes were followed up for 1 year and included evaluation of pain, disability, and quality of life. Disc quality was followed up by magnetic resonance imaging. Results. Feasibility and safety were confirmed and indications of clinical efficacy were identified. MSC-treated patients displayed a quick and significant improvement in algofunctional indices versus the controls. This improvement seemed restricted to a group of responders that included 40% of the cohort. Degeneration, quantified by Pfirrmann grading, improved in the MSC-treated patients and worsened in the controls.

Conclusions. Allogeneic MSC therapy may be a valid alternative for the treatment of degenerative disc disease that is more logistically convenient than the autologous MSC treatment. The intervention is simple, does not require surgery, provides pain relief, and significantly improves disc quality.

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Intervertebral disc degeneration is a very common disease that can lead to axial skeletal pain, radiculopathy, and myelopathy. Combined physical and medical therapies are successful in relieving pain in approximately 90% of the cases. However, the remaining 10% become chronic and generate a serious public health problem, as chronic low-back pain ruins both the life quality and the labor capacity of the patient, and increases use of health services.1,2

The efficacy of the existing treatments is limited (see our recent meta-analysis).3 Surgery is the recommended criterion standard when the back pain chronifies and its analgesic value is beyond question,4 but it has several drawbacks.5,6 Cell therapy has produced exciting results both in vitro and in vivo,7 and animal studies with mesenchymal stem cells (MSC) have been particularly promising.8 On the other hand, in a recent pilot trial in humans, treatment with...
autologous MSC produced strong indications of clinical efficacy, with pain improvement approaching 71% of optimal during the first year. In addition, although disc height was not recovered, water content was significantly elevated.3 This outcome compares favorably with the results of other interventions such as spinal fusion or total disc replacement, suggesting that MSC treatment could be a valid alternative treatment for chronic back pain caused by degenerative disc disease (DDD).

Even though autologous MSCs are an excellent therapeutic option, the need for cell expansion makes the procedure slow and expensive. Allogeneic cells would be logistically, much more convenient, but they may have important drawbacks, the most obvious the possibility of host immune rejection of the transplanted cells. MSC, however, are immune privileged or immune evasive10 and inhibit immune responses in a manner not restricted by the HLA system. As a result, nonmatched MSC are much better tolerated than other cell types. In fact, there are no reports of rejection in animal experiments8,11-14 and studies of transplanted MSC persistence in the host organism show the same values for autologous and allogeneic cells.10,15 In humans, excellent tolerance to allogeneic MSCs has been reported in many clinical trials. For example, in a recent meta-analysis of 87 lupus erythematosus patients, no transplantation-related adverse events were found after 4 years of follow-up.16 Similarly, no transplantation-related adverse events occurred in MSC-treated patients with breast cancer,17 left ventricular dysfunction,18-20 ankylosing spondylitis,21 graft versus host disease,22 and other autoimmune diseases.23 We have demonstrated recently the safety of autologous MSC for treatment of knee osteoarthritis,24 an application very similar to the one proposed in the present paper.

Here, we present a randomized controlled study to assess the feasibility and safety of using good manufacturing practice (GMP)-compliant bone marrow MSCs.3,25 Additionally, we present evidence to suggest that intradiscal injection of allogeneic cells has a therapeutic value. The intervention proposed does not require surgery, does not produce anatomical modifications, and does not hinder further interventions, should they be required.

**MATERIALS AND METHODS**

**Patients and Procedures**

This phase I-II trial was approved by Ethics Committee at Valladolid University Hospital and by the Spanish Agency of Medicines (EudraCT 2012-004444-30). The study was also registered at ClinicalTrials.gov (NCT01860417). The design of the trial was based on our previous one, performed with autologous MSC.7 We recruited 24 patients (17 men and 7 women; mean age ± SE = 38±2 years) with Pfirrmann grade II-IV DDD 26, that had been unresponsive to conventional treatments (physical and medical) for at least 6 months before recruitment. Recruitment was performed between July 2013 and March 2014. Detailed inclusion and exclusion criteria are reported in Table 1. The recruited patients were block randomized by the quality control manager of the cell production facility, who was blinded, to receive either the control or the experimental treatment. The allocation ratio was 1:1. Clinical, analytical, and imaging evaluations were performed to ensure compliance with the inclusion criteria.

| TABLE 1.  |
| Inclusion and exclusion criteria |

**Inclusion criteria**

1. Degenerative disease of 1 or 2 lumbar discs with predominant back pain after conservative treatment (physical and medical) for over 6 months
2. Fibrous ring capable of holding the cell implantation, demonstrated by MRI (stages 2, 3, and 4 of Pfirrmann).
3. Decrease of disc height of more than 20% (radiographic measurement in side image).
4. Absence of spinal infection.
5. Haematological and biochemical analysis with no significant alterations that contraindicate intervention.
6. The patient is able to understand the nature of the study.
7. Informed written consent of the patient.
8. In fertile women, negative pregnancy test result and acceptance of adequate contraceptive methods

**Exclusion criteria**

1. Age, older than 75 yr or younger than 18 yr or legally dependent
2. Allergy to gentamicin, or to bovine, cattle or horse serum.
3. Congenital or acquired diseases leading to spine deformations that may upset cell application.
4. Spinal segmental instability, spinal canal stenosis, isthmus pathology and other conditions that may compromise the study
5. Modic III changes on MRI.27
6. Overweight with body mass index (mass in kg/size in m²) greater than 35 (obesity grade II)
7. Pregnancy or breastfeeding
8. Neoplasia
9. Immunosuppression
10. Hypersensitivity to amide-type local anaesthetics or other known contraindications or interactions of mepivacaine
11. Participation in another clinical trial or treatment with another investigational product within 30 days before inclusion in the study
12. Other conditions that may, according to medical criteria, discourage participation in the study.

Patients were informed of the protocol design before providing written informed consent.

**Follow-Up Controls**

The protocol included 6 visits (V0-V5). The V0 visit involved a final compliance check using the inclusion criteria, performance of the complementary evaluations and tests needed, and scheduling of dates for the next visit. At V1, treatments were administered, either MSCs (25 × 10⁶ MSC in 2 mL of saline per disc) under local anesthesia or sham infiltration of paravertebral musculature close to the affected disc(s) with 2 mL of 1% mepivacaine. The V2-V5 visits (8 days, and 3, 6, and 12 months after implantation) included clinical evaluation and routine analyses, pain evaluation using visual analogue scale (VAS),28 Oswestry Disability Index (ODI),29 and short form-12 (SF-12) life quality questionnaire.30 Outcomes were expressed using a 0% to 100% scale in all cases. Quantitative magnetic resonance imaging (MRI) exploration was performed at V0, V4, and V5. The patients, radiologists, care providers, and persons assessing the outcomes of the assay were blinded after assignment.

**Cell Isolation and Expansion**

Bone marrow was obtained from 5 healthy donors and processed using GMP conditions in the IBGM Cell Production
Unit as described previously.\textsuperscript{3,25} Isolations were carried out with the following parameters (mean ± SD; n = 5, 4 men and 1 woman): bone marrow volume = 105 ± 5 mL, average number of mononuclear cells obtained = 1.23 ± 0.25 \times 10^6, expansion time = 27 ± 2 days, number of MSC injected into each disc = 25 \times 10^6, suspended in Ringer-lactate at 12.5 \times 10^6 cells/mL, and viability greater than 98% ± 1%. A serum sample from each donor was obtained to screen for human immunodeficiency, hepatitis B, and hepatitis C virus by nucleic acid amplification technology.\textsuperscript{31} The cells obtained from each donor were used for 1 to 3 recipients. Immune matching was not attempted.

**Statistics**

Data are reported as mean ± SD or SE, as indicated in each case. Significant differences were assessed by either Student \textit{t} tests, by 2-way repeated-measures analyses of variance, or with appropriate corresponding nonparametric tests. We used GraphPad Instat3 package software version 3.06 (GraphPad Software, La Jolla, CA) for all calculations.

**RESULTS**

Patients

This pilot study included 24 patients (see details in Materials and methods) diagnosed of DDD with preserved external annulus fibrous and persistent low-back pain. Additionally, all 24 patients did not respond to conservative treatment (physical and medical) lasting at least 6 months. The patients had 1 or 2 affected discs, with the lesion located at L1-L2 (n = 1), L2-L3 (1), L3-L4 (3), L4-L5 (18), or L5-S1 (15). They were informed of the protocol design before providing written informed consent. The protocol included 6 visits (V0-V5), as described in Materials and Methods. All patients were treated at the Valladolid University Hospital. No major adverse events occurred. Eleven patients (8 controls and 3 cell-treated) required brief treatments with nonsteroidal anti-inflammatory drugs-type analgesics for minor pains and 2 (1 control and 1 cell-treated) required opioids (morphine sulphate tablets or tramadol).

**Evolution of Pain, Disability, and Quality of Life**

Table 2 summarizes the distribution of pain and disability indexes throughout the observation period. The baseline values of pain and disability were quite homogeneous in the cohort. On average patients felt intense lumbar pain (65 ± 5 in the VAS scale) and had moderate disability (ODI of 29 ± 4) (mean ± SE; n = 24). Both lumbar pain and disability were significantly reduced at 3 months after MSC transplantation, and the improvement was maintained at 6 and 12 months (Figures 1A and C). Compared with the basal level of pain and disability, improvement was statistically significant at all time points except at 8 days (see details in Figure 1, legend). The pattern of improvement between VAS and ODI was parallel and resulted in global displacement of the whole distribution towards smaller values, with a strong decrease of the

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Total sum score of VAS measurements for lumbar pain and ODI

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P25%, P50%, and P75% represent 25th, 50th (median), and 75th percentiles, respectively.

SMD is used here as an estimate of effect size. The correlation between effect size and magnitude of the change are: 0 = null, 0.20 = small, 0.50 = medium, 0.8 = large. Details on effect size can be found in Cohen.\textsuperscript{32}

In all cases, the scale was from 0% to 100%. Measurements performed before cell transplantation (baseline), 8 days, 3, 6 and 12 months afterward (12 months) are shown.

Conf 95 and Conf 99, confidence interval at 95% and 99% significance; min, minimum value; max, maximum value; SMD, standardized mean difference, computed as improvement (baseline value minus value at the end of treatment) divided by the SD of the baseline value; C, control patients (mepivacaine); E, experimental cell-treated patients; N.A., does not apply.
medians (P50% in Table 2; see also Figure S1, SDC, http://links.lww.com/TP/B347). Note that the effect was virtually complete at the third month. In the sham-treated controls the effects at 3, 6, and 12 months were not statistically significant. A fast decrease of pain was detected at the eighth day in the control group, but there was no tendency for further improvement thereafter (Figures 1B and D, and Figure S1B, SDC, http://links.lww.com/TP/B347). The ODI values tended to increase at 3, 6, and 12 months in the control group (Figure 1D).

In Figure 2, we have plotted lumbar pain relief at the end of the treatment, assessed by VAS, as a function of the initial pain score (Figure 2, circles). As shown before, the improvement of the disability index as a function of the baseline ODI value exhibited the same relationship and can be included in the same plot (Figure 2, triangles). The efficacy of the treatment with a slope of 1 is also shown (Figure 1D).

In our previous study with autologous MSCs, we found a slight but significant improvement of the physical component score in the SF-36 quality of life questionnaire. Although this tendency was also observed in the present work, the SF-12 life quality questionnaire did not reveal significant improvements of either the physical or the mental component scores (Table S1; SDC, http://links.lww.com/TP/B347). This is not surprising, because these indices are less sensitive than the pain tests in the inflammatory diseases.

Imaging
MRI was used to assess disc height and water content of the discs. The height of the affected discs decreased by (in mm; mean ± SE) 0.38 ± 0.19 mm the controls (n = 16 discs) and by only 0.04 ± 0.19 mm in the cell-treated patients (n = 17 discs), but the difference was not significant. In neighbor healthy discs (n = 24), the heights decreased by 0.07 ± 0.10 mm (n = 24 discs). These values were not significantly different. Water content of the discs, determined from T2-weighted sagittal images, was measured in the affected disc segment and in the contiguous 3 to 5 segments (see Methods and Orozco et al). In some patients the water content of the affected discs improved after treatment with the MSC with little changes of the normal discs (Figure S2,
extensive and successfully tested for safety (see Introduction),
would be logistically much more convenient than autologous
for these treatments. This trial is, to the best of our knowl-
dge, the first to test therapeutic efficacy of allogeneic MSC
in DDD. Our results show that allogeneic MSC transplantation
is feasible and safe, and does not originate any major adverse
outcome. Occasional mild pain reactions were, if anything,
more frequent in the control than in the cell-treated patients
and responded well to the usual analgesic treatments.

The parallel improvement of pain and disability effected
by the MSC treatment was quick but not immediate; it
reached about 30% of the maximum at 8 days after the inter-
vention, was nearly complete at 3 months and was main-
tained at 6 and 12 months (Figure 1). In the controls not
-treated with MSC the time course was very different. There
was a sudden decrease of VAS during the first 8 days, but
no further improvement along the following year was ob-
erved. As a matter of fact, the disability index worsened
(although not statistically significantly) along the first year
after the intervention. We do not know the reason for this
early improvement of pain in the controls; it could be due
to a placebo effect or be the result from the anesthetic infil-
tration, but in any of these cases, it should also happen in
the cell-treated group, and there is not indication of this extra
fast early improvement in the group of cell-treated patients.

Efficacy of the treatments was quantified by the slope of
the pain relief-initial pain score relationship.28 This gives
values between 0 (no effect) and 1 (perfect treatment). Values
of effect size, a primary measurement of significance (see
Table 2 legend), were also computed. There was a clear anal-
gesic effect of the allogeneic MSC, resulting in an average
28% improvement in pain and disability 1 year after the in-
tervention. This compares with only 15% recovery in the
sham-treated controls (Figure 2). The improvement was

Previous studies have demonstrated that autologous
MSCs provide an excellent therapeutic alternative for
treating DDD; however, allogeneic cells, which have been

FIGURE 2. Pain and disability improvement as a result of MSC
treatment. Improvement 1 year after intervention is plotted as a func-
tion of the initial pain score or disability index.29 Results for the relief of
lumbar pain (circles) and Oswestry disability index (triangles) are all in-
cluded for both, control (open symbols) and cell-treated patients
(filled symbols). The continuous blue line with a slope of 1 represents
the perfect treatment, in which complete pain or disability relief was
achieved. The dotted lines correspond to the linear fit of controls
(black) and treated data (red). The values of the slopes obtained from
the best fit of the data (n = 24; linear regression forced through the
origin) were (mean ± SE): control, 0.15 ± 0.10 (not significantly
different from 0; P = 0.13), and MSC-treated 0.28 ± 0.07 (significantly
different from 0; P < 0.001).

SDC, http://links.lww.com/TP/B347). To homogenize the re-
sults of different patients, the water content values of the af-
ected discs were normalized to the values obtained from the
healthy discs in the same individual; for these purposes, the
density of the affected segments was divided by the average
value of the healthy discs. Finally, the value after the treat-
ment was divided by the baseline value. In the cell-treated
discs the image density increased by (mean ± SE) 22 ± 11 %
at 12 months, compared to only 6% ± 8 % in controls
(Figure S3; Table S2, SDC, http://links.lww.com/TP/B347).
The differences, however, were not statistically significant
(P < 0.07; 1-sample paired t test, 2-tailed value). In our previ-
ous trial with autologous MSC, the difference was very sim-
ilar, 18% increase in water content, but it was statistically
significant because the dispersion was smaller.3 Evolution
of Pfirrmann staging, which takes into account several MRI
disc parameters,28 was clearly different in the control and in
the experimental groups. Results are shown in Figures 3A
and B. In controls (B), there was a deterioration from
(mean ± SEM; n = 20) Pfirrmann stage 3.15 ± 0.15 to stage
3.78 ± 0.16 (P < 0.001; Wilcoxon matched-pairs signed-ranks
test), whereas in the cell-treated patients (A), there was an
improvement from stage 3.68 ± 0.13 to 3.18 ± 0.17 (P < 0.01).

FIGURE 3. Assessment of nucleus pulposus evolution by Pfirrmann
grading. Pfirrmann grading (1 to 5) takes into account the structure of
the disc, the distinction of nucleus pulposus and annulus fibrosus, the
signal intensity and the height of the disc. The values (mean ± SE) at
baseline (open) or 6 months (crosshatched) and 12 months after
-treatment (filled) are shown for control (B) and MSC-treated patients
(A). Comparisons to baseline were performed by repeated measures
ANOVA, Bonferroni multiple comparisons; **P < 0.001.
Importantly, MSCs have also been shown to stimulate cocultured cells to proliferate and synthesize extracellular matrix.49–51 In fact, transplanted MSCs engrafed into the joint in mouse are activated and express Indian hedgehog and other genes. These genes in turn promote expression of collagen II and other chondrogenic genes by host cells.52 Because of these hit and run effects, tracing MSC action may be elusive.

In summary, we propose that cell therapy with expanded allogeneic bone marrow-derived MSC should be considered as a putative treatment for chronic DDD. GMP handling and expansion of these cells is reproducible, and quality control tests are satisfactory. The clinical procedure is feasible and safe and requires minimally invasive intervention without surgery or hospitalization. The procedure results in significant relief of pain and disability, and quantitative MRI evidence suggests partial disc healing. Advantages of allogeneic over autologous treatments include lower cost, higher homogeneity and the possibility of using them in seropositive patients. The healing effects appear to be smaller than those reported for treatment with autologous MSC, but this should be confirmed in future studies designed to directly compare both cell types within the same trial. These studies will track the long-term evolution as well as investigate the anatomical and functional changes that occur in the intervertebral spaces and will increase the number of patients, which is an important limitation of the present study.

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