Percutaneous transplantation of human umbilical cord blood–derived multipotent stem cells in a canine model of spinal cord injury

Laboratory investigation

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Object. The authors describe a method for percutaneous transplantation of human umbilical cord blood (hUCB)–derived multipotent stem cells (MSCs) under fluoroscopic guidance. The investigators then tested whether percutaneous transplantation of hUCB-derived MSCs improved neurological functional recovery after acute spinal cord injury (SCI).

Methods. The authors induced SCI in 10 dogs by percutaneous balloon compression. The 10 injured dogs were assigned randomly to the following groups (2 dogs each): Group 1, evaluated 2 weeks after sham transplantation; Group 2, evaluated 2 weeks after transplantation; Group 3, evaluated 4 weeks after sham transplantation; Group 4, evaluated 4 weeks after transplantation; and Group 5, evaluated 4 weeks after multipoint transplantsations. The dogs with sham transplantation (Groups 1 and 3) received the same volume of saline, as a control. A spinal needle was advanced into the spinal canal, and the investigators confirmed that the end of the spinal needle was located in the ventral part of spinal cord parenchyma by using contrast medium under fluoroscopic guidance. The hUCB-derived MSCs were transplanted into the cranial end of the injured segment in 6 injured dogs at 7 days after SCI.

Results. Two dogs in Group 2 showed no improvement until 2 weeks after transplantation. Three of 4 dogs (Groups 4 and 5) that received cellular transplants exhibited gradual improvement in hindlimb locomotion from 3 weeks after cell transplantation. The CM-DiI–labeled hUCB-derived MSCs were observed in the spinal cord lesions at 4 weeks posttransplantation and exerted a significant beneficial effect by reducing cyst and injury size. The transplanted cells were positive for NeuN, glial fibrillary acidic protein, and von Willebrand factor.

Conclusions. The percutaneous transplantation technique described here can be easily performed, and it differs from previous techniques by avoiding surgical exposure and allowing cells to be more precisely transplanted into the spinal cord. This technique has many potential applications in the treatment of human SCI by cell transplantation. The results also suggest that transplantation of hUCB-derived MSCs may have therapeutic effects that decrease cavitation for acute SCI. (DOI: 10.3171/2009.6.SPINE08710)

KEY WORDS • percutaneous transplantation • spinal cord injury • stem cell • minimally invasive method • dog

Many strategies for the treatment of traumatic SCI are focused on cellular therapies that promote remyelination and functional neurological recovery. These cellular therapies have entailed, at various times, the transplantation of bone marrow stromal cells,3,20 human embryonic stem cell–derived oligoden- drocyte progenitor cells,10 hematopoietic stem cells from bone marrow,18 and neural precursor cells.15 Embryonic stem cell therapy can reduce behavioral defects in damaged and compromised spinal cords in animals and humans. However, embryonic stem cell therapy is complicated by logistical and ethical considerations. Human umbilical cord blood is one of the most useful sources for stem cells because it has more primitive stem cell multilineage differentiation capacity than adult blood, including the capacity for neural cell differentiation.31

Abbreviations used in this paper: GFAP = glial fibrillary acidic protein; hUCB = human umbilical cord blood; MNC = mononuclear cell; MSC = multipotent stem cell; PBS = phosphate-buffered saline; SCI = spinal cord injury; SEP = sensory evoked potential; vWF = von Willebrand factor.

This article contains some figures that are displayed in color online but in black and white in the print edition.
Stem cells in umbilical cord blood are more immature than other adult stem cells and do not induce a strong rejection response. Furthermore, the traditional ethical dilemmas are circumvented. Due to their primitive nature and capacity to develop into nonhematopoietic cells of various tissue lineages, hUCB-derived MSCs represent a potentially useful source for cell-based therapies after SCI.

There are various approaches to transplantation, including direct local administration, intravenous administration, and CSF injection. Recent animal experiments demonstrate that delivery of stem cells into the posttraumatic spinal cord cavity promotes a clear and progressive functional recovery, significantly superior to the recovery obtained through intravenous administration. Problems associated with intravenous cell transplantation include reliance on injury-mediated opening of the blood-brain barrier to allow cells access to the CNS parenchyma. Some investigators have injected cells directly into the parenchyma of the contused spinal cord or caudal and rostral to the injured spinal cord. Although these methods might be acceptable, direct injection requires anesthesia and surgical reexposure of the spinal cord, which increase the risk of infection and damage to surrounding tissues.

To date, rats have been the animals most commonly used for studies of stem cell therapy because they are economical to obtain and maintain, and are readily available. However, the spinal cord of the rat is far smaller than that of humans, and there the rat model had its limitations. In light of these drawbacks, we investigated an alternative percutaneous method of stem cell injection aided by fluoroscopic guidance in a canine model of SCI. We then tested whether percutaneous transplantation of hUCB-derived MSCs improved neurological functional recovery after acute SCI.

**Methods**

**Model of SCI**

Ten beagle dogs of either sex, each weighing between 7 and 10.7 kg, were used in this study. Animal experiments were approved by the Institutional Animal Care and Use Committee at the College of Veterinary Medicine at Konkuk University. The dogs were maintained according to the Guide for the Care and Use of Laboratory Animals from the Institute for Laboratory Animal Research, National Institutes of Health.

All procedures followed the methods described previously. Briefly, after general endotracheal anesthesia was induced, dogs were positioned in the right recumbent position on the operating bed. The lumbar epidural space was entered with a spinal needle via the lumbosacral joint, with the aid of fluoroscopic guidance. A guidewire was then introduced through the needle, and the spinal needle was removed. The introducer and dilator were inserted into the epidural space over the guidewire, and the dilator and guidewire were then removed, leaving the introducer in place. Vertebral bodies that contain the cauda equina are L5–Cd5 in dogs. A balloon catheter was then inserted into the epidural space through the introducer and advanced under fluoroscopic guidance until it was between L-2 and L-3. The balloon was then inflated with half-strength iohexol (Omnipaque, Amersham Health) and an inflation device until it reached the desired final volume (from 0.7 to 0.8 ml). The position and shape of the balloon were confirmed by fluoroscopy by using contrast medium, and the balloon was left in place for 60 minutes. The balloon was then deflated and removed. Postprocedural analgesia was provided by epidural injections of medetomidine (5 µg/kg) and bupivacaine at 0.5 mg/kg, along with an injection of intramuscular buprenorphine at 10 µg/kg. Manual bladder expression was performed daily to treat dysuria, and cephalexin was administered orally at 30 mg/kg. Soft pads and a sling were used to prevent pressure sores.

**Evaluation of SEPs**

After induction of general anesthesia, for scalp SEP recording, Cz electrodes (placed ~ 5 mm behind the bregma on the midline) were referenced to Fz electrodes (placed ~ 5 mm in front of the bregma on the midline), both of which were monopolar needle electrodes. For lumbar potential recording, an L-4 (placed on the 4th lumbar vertebra) monopolar disc electrode was referenced to L-3 (placed on the 3rd lumbar vertebra). For ground, a needle monopolar electrode was used and inserted into the hip. The left tibial nerve was stimulated. Stimulus intensity was kept at a level that resulted in slight twitching of hindlimb digits. The SEP signals were recorded (Sierra Wave, 2006; Cadwell Laboratories, Inc.) immediately before SCI, at 10 minutes after compression, at 10 minutes after decompression, and before planned death. Three hundred stimuli were delivered at a rate of 2.82 Hz and averaged. The stimulus intensity was 1 mA, and the sweep time was 30 msec.

**Isolation, Expansion, and Immunophenotyping of hUCB-Derived MSCs**

Freshly collected hUCB samples from full-term deliveries were acquired after obtaining written informed donor consent. The MNCs were isolated from a low-density mononuclear fraction (MNC < 1077 g/ml) by Ficoll-Paque Plus (GE Healthcare AB). Total MNCs were cultured in DMEM low-glucose medium (Gibco BRL) containing 20% fetal bovine serum (Gibco) including basic fibroblast growth factor (10 ng/ml), stem cell factor (10 ng/ml), 100 U penicillin, 1000 U streptomycin, and 2 mM l-glutamine (Gibco) and plated in T-25 flasks at a concentration of 5 x 10³ cells/cm². The hUCB cells were maintained at 37°C in an incubator containing a 5% CO₂ humidified atmosphere. Culture medium was replaced every 4 days. The MSCs from attached cells were passages by trypsinization (0.005% trypsin/EDTA; Gibco) on reaching 80–90% confluence at 5 x 10⁴ cells/cm² in the T-25 flasks.

At the second or third passage, spindle-shaped homogeneous MSC populations were trypsinized and examined by phycoerythrin-conjugated anti-CD29 and anti-CD166 as well as fluorescein isothiocyanate–conjugated...
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anti-CD14, anti-CD34, anti-CD44, anti-CD105, and anti-CD166 (BD PharMingen). Flow cytometry analysis was performed as described previously.11

Cell Preparation Labeling

The CM-DiI (CellTracker CM-DiI, Molecular Probe) was diluted to 1 µg ml⁻¹ with PBS. The 10⁶ hUCB-derived MSCs were incubated in 1 ml of CM-DiI solution for 5 minutes at 37°C, followed by an additional 15 minutes at 4°C on ice. After labeling, the hUCB-derived MSCs were washed twice with PBS and resuspended in 300 µl of saline. Then the hUCB-derived MSCs were washed and re-suspended in PBS to a concentration of 10⁶ cells/300 µl.

Percutaneous Transplantation Under Fluoroscopic Guidance

Transplantation of hUCB-derived MSCs was performed 7 days after the SCI procedure. The 10 injured dogs were assigned randomly into the following groups (2 dogs in each group): Group 1, evaluated 2 weeks after sham transplantation; Group 2, evaluated 2 weeks after transplantation; Group 3, evaluated 4 weeks after sham transplantation; Group 4, evaluated 4 weeks after transplantation; and Group 5, evaluated 4 weeks after multi-spot transplantations. The dogs with sham transplantation (Groups 1 and 3) received the same volume of saline as a control (Table 1).

Dogs were anesthetized by intravenous administration of propofol at 6 mg/kg. Anesthesia was maintained by delivering 1.5% isoflurane through an endotracheal tube. The animals were positioned in the right recumbent position on the operating bed, and each dog’s back was flexed. With the paramedian approach, the spinal needle (26 gauge) was inserted slightly caudolateral to the spinous process of the lumbar vertebra, and directed cranioventrally at a 45° angle, through the interarcuate space (Fig. 1B).

A spinal needle was advanced into the spinal canal between L-1 and L-2, and the stylet was removed. We confirmed that the end of the spinal needle was located within the spinal cord parenchyma by using contrast medium (0.1–0.2 ml) under fluoroscopic guidance (Fig. 1A). The needle’s tip was placed in the ventral part of the spinal cord. While the syringe was attached and cells were injected, the correct needle placement was confirmed under fluoroscopy. When needle displacement was observed, it was immediately corrected. The needle was kept in place and horizontal movements were prevented to minimize damages. These procedures provided accuracy of location of the needle tip for cell transplantation.

Injections of 10⁶ CM-DiI–prelabeled hUCB-derived MSCs suspended in 0.3 ml of saline were performed directly into the cranial end (L1–2) of the injured segment (Groups 2 and 4). In multispot transplantation, injections of 10⁶ hUCB-derived MSCs suspended in 0.1 ml of saline without labeling were performed directly into 3 separate spinal cord regions of the dogs (Group 5. Dogs 9 and 10); that is, cranially (L1–2), and caudally (L3–4) to the injured segment, and directly to the injured segment (L2–3). As a result, the total volume was 0.3 ml, the same as the other test group. The injection was performed over a 2-minute period to avoid additional trauma. The spinal needle was left in place for 3 minutes after injection and was then withdrawn. The entire procedure took between 10 and 15 minutes.

Behavioral Testing

All behavioral tests were digitally videotaped and converted to computer video files. Three independent examiners who were blinded to the dog’s treatment status observed the hindlimb movements of each dog and scored the locomotor function according to the modified Tarlov score.28 Applied to each hindlimb, this scale is graded in 6 levels, as follows: 0, no movement; 1, minimal voluntary joint displacement; 2, limb movement, but will not bear weight; 3, animal able to stand up but unable to walk; 4, walks with deficit; and 5, normal gait.

Deep pain sensation (nociceptive reaction) was also assessed. The presence of deep pain sensation was determined by applying pressure across the nail bed of each digit; presence of sensation was noted when the dog was mentally aware of the stimulus (for example, cried out or looked around). Evaluation of behavior and deep pain sensation was performed before SCI, and at 1, 7, 14, 21, 28, and 35 days after SCI.

Histological and Immunohistochemical Analysis

Dogs were deeply anesthetized with 12 mg/kg of intravenous propofol and then killed by administering an overdose of intravenous potassium chloride at either 2 (Groups 1 and 2) or 4 weeks (Groups 3, 4, and 5) after cell transplantation.

**TABLE 1: Physical parameters and locomotor evaluation according to the modified Tarlov score after hUCB-MSC transplantation in 8 dogs**

<table>
<thead>
<tr>
<th>Group</th>
<th>Dog No.</th>
<th>BW (kg)</th>
<th>Transplantation Category</th>
<th>1 Wk</th>
<th>2 Wks</th>
<th>3 Wks</th>
<th>4 Wks</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1</td>
<td>9.8</td>
<td>ND</td>
<td>0</td>
<td>0</td>
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<td>—</td>
</tr>
<tr>
<td>2</td>
<td>7.2</td>
<td>ND</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>3</td>
<td>5</td>
<td>8.9</td>
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<td>5</td>
<td>9</td>
<td>10.1</td>
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<td>0</td>
<td>0</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>10</td>
<td>10.7</td>
<td>multi</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>2</td>
</tr>
</tbody>
</table>

* Two of the 10 dogs were controls. All Tarlov scores were 0 before transplantation. Abbreviations: BW = body weight; multi = multispot transplantation; ND = not done; S = single-spot transplantation.
transplantation or saline injection. The T13–L4 expanses of the spinal cords in these dogs were harvested without perfusion. Specimens were fixed for 12 hours in 10% neutral buffered formalin at 4°C, then embedded in paraffin. Spinal cord tissue blocks were cut in the sagittal or transverse plane at 4-µm thickness. Sections were collected on gelatin-coated slides. The sections were examined with a fluorescence microscope (BX51 and DP71, Olympus) with excitation at 510–550 nm and emission at 460–490 nm. Preparation of specimens with H & E staining was performed for histological evaluation.

For immunohistochemical studies, tissue sections were washed in PBS and treated with 1% H2O2 to block endogenous peroxidase. Nonspecific binding was blocked through incubation with a blocking solution (3% bovine serum albumin in PBS solution) for 1 hour at room temperature. The sections on each slide were then incubated in 10% bovine serum albumin with anti-NeuN (1:100, monoclonal; Chemicon), anti-GFAP (1:500, polyclonal; Dako), and anti-vWF (1:1000, polyclonal; Chemicon) at 4°C overnight. Primary antibodies were removed, and the slides were washed 3 times with PBS. Secondary antibodies (1:200, goat anti–mouse or goat anti–rabbit; Alexa 488, Molecular Probes) were then added and incubated for 1 hour. These were then removed, and the slides were washed 5 times with PBS.

Immunofluorescence colocalization studies were performed with a laser-scanning confocal microscope (FV-1000 Spectral, Olympus) to assess differentiation of the transplanted CM-DiI–prelabeled hUCB-derived MSCs into glial cells, neurons, and endothelial cells of blood vessels in the injured spinal cord.

Fig. 1. A: Fluoroscopic image demonstrating the spinal needle placement between L1 and L2 under fluoroscopic guidance. The 0.3-ml volume of contrast media was detected on the tip of the needle. B: Photograph of spine model showing transplantation method. The tip of the needle was inserted in the spinal canal.

Fig. 2. Recording of SEPs in Dog 4, in which SCI had been induced. After spinal cord compression, the peak amplitude at the scalp deteriorated, with an eventual complete loss of the peaks, whereas the peaks of L-4 were maintained after SCI. Div = division.
Results

Immunophenotyping of hUCB-Derived MSCs

In agreement with Gang et al.,
10 ex vivo expanded hUCB-derived MSCs collected after the second or third passage did not express CD14, CD34, CD45, or HLA class II antigen in this study. However, the MSC population was characterized as positive for CD29, CD44 (the hyaluronate receptor), CD105 (endoglin/Src homology domain–2), CD106, CD166 (activated leukocyte cell adhesion molecule–1), and HLA class I molecule based on results of flow cytometry. This result was in agreement with bone marrow or hUCB-derived MSC immunophenotypes previously reported by other investigators.

32,34 This means that hematopoietic cells were removed from the MSC population by the second or third passage.

Intraspinal Transplantation Under Fluoroscopic Guidance

The labeled cells were successfully transplanted into the spinal cord parenchyma with the aid of fluoroscopic guidance. When a small volume of contrast medium (0.1–0.2 ml) was injected into the spinal canal, it stayed around

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**Fig. 3.** Photomicrographs of H & E–stained sagittal spinal cord sections obtained 4 weeks after intraspinal hUCB-derived MSC transplantation (A) and 4 weeks after SCI only (B). The dotted lines mark the boundary of the spinal cord damage, which consisted of hemorrhage and diffuse parenchymal necrosis with vascular formations. A: The cavitation of spinal cord in Dog 7, in which the hUCB-derived MSC transplantation had expanded to the site of injury between the L-2 and L-3 levels. B: In the spinal cord of Dog 5 (SCI only), the region of cavitation extended between the L-1 and L-3 levels. Bar = 3 mm.

**Fig. 4.** A: Sagittal section obtained at 4 weeks after injection into the spinal cord of Dog 7 is shown on fluorescence microscopy. The CM-DiI–labeled cells migrated into the injured spinal cord from the injection site. B: Few cells are shown at the injection site under high magnification. C: A number of Dil-labeled cells (arrowheads) are shown at the injury site under high magnification. Ca = caudal end; Cr = cranial end. Boxes in panel A labeled “b” and “c” correspond to panels B and C, showing the same area at higher magnification. Bar = 300 µm.
the central canal of the spinal cord (Fig. 1). Needle placement was reconfirmed throughout the procedure with contrast medium injection.

Recovery of Hindlimb Function

Ten dogs underwent successful balloon compression. The SCI procedure produced complete hindlimb paralysis and loss of nociceptive reflex response in all dogs. The modified Tarlov score dropped from 5 to 0 on the 1st day after the SCI procedure and did not improve in the week until transplantation.

Six dogs (Groups 2, 4, and 5) received hUCB-derived MSC transplants on Day 7 after SCI. Two dogs in Group 2 showed no improvement until 2 weeks after transplantation. Three of 4 dogs (Groups 4 and 5) that received cellular transplants exhibited gradual improvement in hindlimb locomotion from 3 weeks after cell transplantation. The modified Tarlov score of these dogs increased to 2 or 3 points at 4 weeks after transplantation. The modified Tarlov score in the other dog in Group 4 remained at 0 until planned death. In the SCI-only dogs (Groups 1 and 3), the modified Tarlov scores remained at 0 points without any obvious improvement, and urinary dysfunction continued for 5 weeks until the dogs were killed. These dogs moved using only their forelimbs, and there were neither voluntary hindlimb movements nor nociceptive reaction (Table 1). Figure 2 shows the SEP of a dog before and after the SCI procedure. After spinal cord compression, the scalp’s peak amplitude deteriorated, with an eventual complete loss of the peaks, whereas the peak amplitude of L-4 was maintained after SCI. The complete loss of the scalp peak did not recover on SEP testing until planned death.

Histological Evaluation and Immunohistochemical Analysis

Hemorrhage and diffuse parenchymal necrosis with vascular formations were detected in both the only SCI-only dogs (Group 1) and the dogs that received transplanted cells (Group 2) between L-2 and L-3 of spinal cord at 2 weeks after transplantation. However, at 4 weeks after transplantation, 4 dogs with cell transplantation (Groups 4 and 5) showed that cavitation had remained between the L-2 and L-3 levels (Fig. 3A). However, the spinal cords of 2 dogs with SCI only (Group 3) showed a region of cavitation that extended between the L-1 and L-3 levels (Fig. 3B).

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**Fig. 5.** Confocal laser micrographs of the injured region of the spinal cord 4 weeks after transplantation. Double-positive colocalized areas for CM-Dil–labeled MSCs/NeuN (panels A–C), MSCs/GFAP (panels D–F), and MSCs/vWF (panels G–I) are indicated in the confocal microscopic images. Merged images exhibiting CM-Dil (red) and immunofluorescence (green) are shown (panels C, F, and I). The labeled cells show partial colocalization (arrows). Bar = 20 µm.
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Fluorescent microscopy of spinal cord sections showed bright fluorescence from the lesion cavity (Fig. 4). The CM-DiI–labeled cells were localized throughout the injury site, whereas no CM-DiI–labeled cells were observed at the site of transplantation. Fluorescence microscopy revealed a number of DiI-labeled cells in the lesion. All the CM-DiI–labeled MSCs were located in or around the injured parenchyma (Fig. 4C); a few cells were observed on the ventral and caudal sides of the transplantation sites (Fig. 4B), indicating that the hUCB-derived MSCs migrated to the injured site.

On confocal microscopic analysis, CM-DiI–labeled hUCB-derived MSCs were also detected at the injured site at 2 and 4 weeks after transplantation. The prelabeled MSCs were not detected at the site of transplantation. We observed the colocalization of the CM-DiI–labeled cells with NeuN (Fig. 5A–C) or GFAP (Fig. 5D–F) in the epicenter of the injured spinal cord. Compared with the control group, there was an increase in CM-DiI–labeled cells expressing the marker of vWF. These findings were demonstrated on merged reconstructed images (Fig. 5C, F, and I) by the FiuOVIEVE program on the confocal microscope system. The injured spinal cords of dogs in the control group showed negative results on immunohistochemical analysis.

Discussion

Many animal experiments have demonstrated that the delivery of stem cells into posttraumatic spinal cord cavities promotes progressive functional recovery, and that these outcomes are significantly better than those achieved by intravenous administration.3,30 The authors of most studies have demonstrated some cellular homing into the pathological CNS tissues after intravenous injection.12 However, some authors have demonstrated that cells delivered intravenously are likely to get trapped in other organs such as the liver, lung, and spleen.27

Recently, Bakshi and colleagues3,4 reported a lumbar puncture technique for transplantation in an SCI model. They confirmed the proper placement of the needle in the lumbar subdural space based on the following 3 signs: 1) a feeling of “give” at the time of entry; 2) a tail flick; and 3) presence of CSF in the needle hub. This method is more appropriate in rats than in humans, because a tail flick sign obviously does not apply to humans. Concerning the third sign, it is technically more difficult to collect CSF from lumbar subarachnoid space than from the cerebellomedullary cistern in canines.2 Furthermore, cell injection occurred at the lumbar level, whereas the confused spinal cord was at the midthoracic level.3,4 In thoracolumbar lesions characterized by spinal cord swelling over several segments, lumbar CSF may be difficult to obtain.27

Although these investigators’ methods are comparable to ours, our method offers several advantages. We injected a small amount of contrast medium to confirm correct intraspinal location of the spinal needle under fluoroscopy. This technique prevented inadvertent movements of the needle during stylet removal or during subsequent attachment of a syringe for transplantation. It also reduces the chance of the needle tip puncturing the bottom of the vertebral canal and penetrating the abdominal cavity or colon.

Our method is easily performed in 15 minutes under fluoroscopic monitoring at the bedside, after injection of a local anesthetic. Although the dog is the only animal in which this technique has been tried, we know of no reason why other vertebrate animals, including humans, should not be able to undergo the procedure. Transmedullary passage of the needle is necessary for transplantation, and the damage of the spinal cord is inevitable in this procedure. There is a small risk of spinal cord damage secondary to needle insertion. This risk exists also in the lumbar puncture technique that Bakshi et al.3 reported, and in other studies in which subarachnoid space30,33 or intramedullary injection were used.14,18 However, the potential therapeutic benefits of our transplantation method outweigh this risk. In addition, there is no damage to surrounding tissues, because there are no surgical procedures involved, which are more harmful to patients.

We believe that our transplantation methods will have major implications for cell transplantation in humans with SCI. Furthermore, we wanted to confirm whether multipot transplants are possible with our methods. “Multipot” means that our technique allows multiple transplantations of therapeutic cells without a surgical procedure. Multiple transplantations into the injured spinal cord were previously described in studies of human patients9 and rats.4 The different outcomes might have been influenced by the difference in transplantation method; however, in this study the data were not sufficient to reach a firm conclusion. These results will be in a subsequent report.

We were able to transplant hUCB-derived MSCs into a dog model of SCI without the need for immunosuppressive treatments. This might be explained by the hypoinmunological features of MSCs, such as the absence of HLA class II expression, inhibition of T-cell response, and B-lymphocyte attenuation.29,31 Several in vitro and in vivo immunosuppressive properties of MSCs have been reported. Adult MSCs and hUCB-derived MSCs are not immunostimulatory in vitro and fail to induce proliferation of allogeneic lymphocytes.20,21,29,31 No immunoreactivity was observed when patient lymphocytes were reexposed to the graft in vitro, indicating that MSCs can be tolerated when transplanted across the major histocompatibility complex barrier in humans.21 There is indirect support for an immunosuppressive effect of the MSC-like cell derived from umbilical cord. Chrisman17 and Kim et al.15 have transplanted UCB-derived cells xenogenically in nonimmun-suppressed hosts, with no observation of frank immune rejection. As Jeong et al.13 have demonstrated, hUCB-derived MSCs have differentiation capability. The hUCB-derived MSCs may facilitate the healing process in SCI by neuronal cell differentiation15 or by other effects such as releasing cytokines, chemokines, and neurotrophic factors.10,18,26 The UCB-derived MSCs also differentiate along an endothelial lineage in vitro,11 and have therapeutic effects in vasculature-directed regenerative medicine.17 Previous studies support our findings that transplantation of hUCB-derived MSCs into...
animals with SCI can promote neurovascularization and can differentiate into neurons and astrocytes; these cells survived for at least 1 month without immunosuppressive therapy.

It may be that the release of trophic factors by hUCB cells is sufficient to support damaged tissues of the spinal cord or brain, which may subsequently allow behavioral recovery.23 In the present study, histological examination demonstrated that hUCB-derived MSC transplantation can also decrease cavitation and hemorrhage. However, the detailed mechanisms of MSC immunogenic activity and of specific processes in the survival of injected MSCs are still unclear. Nevertheless, the present study reveals that hUCB-derived MSCs may exert a beneficial effect when transplanted into the injured spinal cord. When we compared the cavitation size between control and cell transplant groups 4 weeks posttransplantation, we found that hUCB-derived MSC transplantation can also decrease cavitation in cell transplant groups. First of all, animals in control groups that did not receive transplanted cells showed no improvement, obviously. However, the animals in the cell transplant groups showed functional recovery.

We recently described a percutaneous SCI model producing irreversible paraplegia in 7 dogs.23 Because laminectomy alone has the effect of decompression, an SCI model including laminectomy is inappropriate for evaluating an intact therapeutic regimen. We did not undertake any decompressive surgical procedure in the present study. This strongly supports the idea that clinical improvement is attributable to the effects of transplantation. Also, in cell transplant groups, the hUCB-derived MSCs survived well in injured spinal cord, and CM-DiI–labeled cells were coexpressed with NeuN, GAFP, and vWF in immunohistochemical findings. Our results indirectly support the hypothesis that these cells may differentiate into functional cells that have beneficial effects on recovery after SCI. However, the number of dogs included in this study was small, and variability was noted in the recovery of motor function over the 4-week test period. Further investigation and longer observation time are needed for evaluation of locomotor function. The specific reasons for functional recovery of SCI remain to be fully explained, and detailed experiments are required.

Conclusions

We transplanted hUCB-derived MSCs into the spinal cord of a group of dogs to evaluate the role of those cells in an SCI model. We also established that percutaneous transplantation under fluoroscopic guidance is easily applied in SCI, is minimally invasive, and has a low risk of infection. Our method allows for effective delivery of multiple therapeutic doses—including drug delivery—while the spinal dura mater remains intact, and has many potential clinical applications in humans for cell transplantation to treat SCI. Our results also suggest that transplantation of hUCB-derived MSCs may have therapeutic effects that decrease cavitation for acute SCI.

Disclaimer

The authors do not report any conflict of interest concerning the materials or methods used in this study or the findings specified in this paper.

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Manuscript submitted October 17, 2008.
Accepted June 29, 2009.

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