Functional Outcome of Bone Marrow Stem Cells (CD45+/CD34-) After Cell Therapy in Acute Spinal Cord Injury: In Exercise Training and in Sedentary Rats


ABSTRACT

Background. Cell therapy and exercise training may be options for spinal cord regeneration. Our objective was to evaluate the functional effects of autologous bone marrow stem cell (CD45+/CD34-) transplantation in acute spinal cord injury in exercise training and in sedentary rats.

Materials and Methods. Fifty-five adult male Wistar rats underwent spinal cord contusion by Impactor (NYU). Locomotor rating scale was performed every 48 hours for 48 days. Animals with scores ≤12 were randomly divided into 4 groups: sedentary without parenchymal cell infusion; sedentary with parenchymal cell infusion; swimming training without parenchymal cell infusion; and swimming training with parenchymal cell infusion. Bone marrow stem cells were isolated by puncture-aspiration of the bone marrow and density gradient (d = 1.077). The animals underwent a 60-minute swimming session 6 times/week supporting an overload of 3% of body weight for 6 consecutive weeks. Comparisons between the groups in relation to differences between the beginning to the end of scores used the nonparametric Bonferroni test and post-hoc Mann-Whitney U test to identify significance.

Results. Forty-two rats that obtained scores ≤12 underwent therapy with 9 animals in each of the 4 groups as completors (n = 36). There was significance (P ≤ .008) for sedentary without parenchymal cell infusion vs swimming training with parenchymal cell infusion.

Conclusion. The combination of bone marrow stem cell therapy (CD45+/CD34-) and exercise training resulted in significant functional improvement in acute spinal cord injury.

SPINAL CORD INJURY (SCI) is the cause of major clinical, social, and economic problems. The incidence has been estimated to be between 20 and 50 cases per million per year. It is lower than the incidences of other diseases and traumas. SCI usually involves young patients. The physiopathology of spinal cord trauma is determined by 2 basic conditions: the trauma itself, involving either cellular death or electrolyte, metabolite, and enzyme release, and the cascade of acute inflammation with swelling, ischemia, and reperfusion as a secondary neuronal injury. Research into regeneration of spinal cord nervous tissue uses cell transplantation and seeks to minimize secondary damage by inflammation with drugs. Both strategies seek to improve the quality of life of these patients. Cell transplantation has become the most promising treatment for neurodegenerative diseases or central nervous system injuries such as spinal cord trauma. Our objective was to evaluate the functional effects of autologous bone marrow stem cell...
Blood lactate concentrations.

**MATERIALS AND METHODS**

**Animals**

Fifty-five adult male Wistar rats underwent spinal cord contusion injury by Impactor (NYU). All experiments involving the animals were conducted in conformance with the policy statement of the American College of Sports Medicine on Research with Experimental Animals. Male Wistar rats of 90 days old and weighing an average of 350 g received commercial chow and water ad libitum. The animals were housed in collective cages (2 rats per cage, after the SCI). All rats were kept on 12-hour light-dark cycles.

**Spinal Cord Injury**

The rats were evaluated according to the Multicenter Animal Spinal Cord Injury Study. The animals received ketamine and xylazine (50 and 10 mg/kg IP, respectively) for anesthesia for a T9–T10 laminectomy, preserving the dural sac. Fifty-five rats suffered a contusion injury with a 2 mm² bar weighing 10 g falling from a 25 mm height, above the cord. This procedure produces graded lesion severity with the NYU Impactor model for data analysis. All animals received cefazolin (1 mg/d IP) at the moment after the surgical procedure, at 24 hours, and at 48 hours after lesion.

**Isolation Cells**

Collections of mononuclear cells obtained by puncture-aspiration of blood bone marrow from the iliac crests underwent isolations using a density gradient, Ficoll-Hypaque (d = 1.077), according to Boyum. Flow cytometric analysis (FACScalibur, Becton Dickinson, Franklin Lakes, NJ, United States) was performed to validate the bone marrow origin of the stem cells. Immunophenotyping for CD34 was performed with a commercially available kit (Stem Kit, Beckman Coulter, Krefeld, Germany) as a single-platform method according to the International Society of Hematotherapy and Graft Engineering (SHAGE) guidelines. This kit consists of an anti-CD34-PE, and a respective isotype-matched control-PE MAb. The conjugated MAbs are already provided in defined combinations ready to use. All flow cytometric analyses were performed in duplicate, and the mean values were calculated from the results.

**Exercise**

All rats were adapted to the water before the beginning of the experiment. The adaptation consisted of keeping the hot in the water at 31°C ± 1°C for 10 minutes in 3 different levels of water columns: first day, 20 cm; second day, 30 cm, and last day, 40 cm.

**Acute Exercise-Test Protocol**

Rats underwent swimming exercise in 1 tank of 80 × 40 × 50 cm, subdivided into 2 compartments. Each animal participated in 2 experimental tests: one at the beginning of the exercise training and the other at the end of the 42nd day. Each test consisted of continuous swimming for 10 minutes without a load to analyze blood lactate concentrations.

**Blood Lactate Analysis**

Blood samples (25 μL) were collected from a cut at the tail tip of the end of the exercise test. The rats (n = 18) were quickly dried with a towel immediately before the test. This test was analyzed in the 2 groups: swimming training without cells and swimming training with cells. The lactate concentrations were determined in a lactate portable analyzer (Accutrend).

**Exercise Training**

The animals were trained to swim 60 min/d, 6 d/week, with an overload of 3% body weight, for 6 weeks. The exercise sessions were done at the same hours, around noon. Incremental exercises were obtained by adding progressively heavier loads in relation to body weight, attached to the animal’s chest or tail.

**Locomotor Scale**

The Basso, Beattie, and Bresnahan (BBB) locomotor rating scale was done after injury and after therapy every 48 hours for 46 days by 2 examiners as a 4-minute double-blind test.

**Cell Therapy**

At 48 hours after spinal cord injury, the animals with scores ≤12 were randomly divided into 4 groups: group one (sedentary without parenchymal cell infusion); group two (sedentary with parenchymal cell infusion); group three (swimming training without parenchymal cell infusion); and group four (swimming training with parenchymal cell infusion). The treatment included 5 × 10⁶ cells injected directly into the spinal cord, using a 15 μL Hamilton syringe (1701 LT, Hamilton Bonaduz AG).

**Statistical Analysis**

To evaluate functional outcomes, the locomotor rating scale (BBB) compared groups using the nonparametric Mann-Whitney U test (P ≤ .05) with Bonferroni correction (P ≤ .008). Blood lactate concentrations were performed before and after swimming training. The analyses were performed by paired Student t test (P ≤ .05).

**RESULTS**

To evaluate functional outcomes, statistical analyses showed significance at baseline and at the final point of exercise training (P ≤ .008; Bonferroni correction) for animals that had swimming training with cells (Table 1). For blood lactate concentrations, statistical analyses demonstrated significance for the swimming training with cells cohort (P ≤ .05; Table 2).

**DISCUSSION**

Previous studies have shown that exercise or transplantation alone has attenuated the atrophy and loss of movement, if implemented shortly after injury. In this work, we demonstrated that the combination of bone marrow stem cell therapy (autologous mononuclear fraction) and exercise training resulted in significant functional improvement in acute spinal cord injury. This observation may be explained by release of factors from exercising muscles that stimulate neuronal outgrowth such as neurotrophin. Nevertheless, the mononuclear fraction of bone marrow stem cells also had progenitors of anti-inflammatory cells since anti-apoptotic molecules are produced by mesenchymal...
stem cells (MSC). MSC are observed among up to 0.01% of the mononuclear fraction. These cells may induce a suppressive local microenvironment through production of prostaglandins and interleukin-10 as well by expression of molecules which deplete the local milieu of tryptophan. The purpose of the adaptation was to reduce the stress without, however, promoting physical training adaptation. The use of swimming rats as a model of exercise presents advantages over treadmill running, since swimming is a natural ability of rats. Blood lactate concentration analysis confirmed the locomotor efficiency. These results suggested that an improved locomotor rating scale (BBB) reduced metabolic consumption.

The BBB scale can distinguish differences in neurological outcomes of rats given injuries at various trauma doses; minimal recovery was noted when using the BBB scale in rats with severe injuries. The histological results are ongoing, but the available data suggest that the beneficial effects could be due to actions on secondary damage: namely, more to preserve existing cells than foster regeneration with development of new neurons.

ACKNOWLEDGMENTS
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REFERENCES

Table 1. BBB Scale: Baseline, Final, and Difference

<table>
<thead>
<tr>
<th>Variable</th>
<th>Sedentary Without Cells (n = 9)</th>
<th>Sedentary With Cells (n = 9)</th>
<th>Swimming Training Without Cells (n = 9)</th>
<th>Swimming Training With Cells (n = 9)</th>
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<tbody>
<tr>
<td>Baseline BBB scale</td>
<td>2.89 ± 2.42</td>
<td>2.22 ± 2.95</td>
<td>1.89 ± 2.26</td>
<td>1.67 ± 2.06</td>
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<td>Final BBB scale</td>
<td>10.33 ± 3.32</td>
<td>11.22 ± 4.71</td>
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<td>17.58 ± 2.83</td>
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<td>Difference (baseline vs final BBB)</td>
<td>7.44 ± 3.42*</td>
<td>9.00 ± 2.78</td>
<td>11.22 ± 2.49</td>
<td>15.89 ± 2.76*</td>
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<td>*Bonferroni test (P ≤ .008).</td>
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Table 2. Blood Lactate Concentration Analysis (mmol/dL)

<table>
<thead>
<tr>
<th>Group</th>
<th>Before Therapy</th>
<th>After Therapy</th>
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<tr>
<td></td>
<td>Mean  SD</td>
<td>Mean  SD</td>
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<tr>
<td>Swimming training without cells</td>
<td>5.31  0.40</td>
<td>4.98  0.45</td>
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<tr>
<td>Swimming training with cells</td>
<td>5.40*  0.44</td>
<td>4.31*  0.40</td>
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<td>*Paired t test (P ≤ .05).</td>
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849