Improving Sperm Viability After Spinal Cord Injury Using Hyperbaric Therapy

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BACKGROUND: **Infertility is one of many complications of spinal cord injury (SCI) in male patients, who are often at the peak of their reproductive life. This study evaluated effects of hyperbaric therapy (HT) on quality of sperm of rats with SCI and correlated the findings with histologic analysis of the testicles.**

METHODS: **This experimental study comprised 18 rats that were submitted to SCI with a MASCIS Impactor and randomly allocated to either a HT or a control group. Testicular biopsies were performed on the first and 28th day of the study; 4 parameters were evaluated: concentration of sperm per mL, number of round cells per field, number of inflammatory cells per field (peroxidase [Endtz] test), and sperm viability (hypo-osmotic swelling test).**

RESULTS: **There was no difference in sperm concentration between the HT group ($P = 0.41$) and control group ($P = 0.74$) during 28 days. From day 1 to day 28, sperm viability decreased twice as much in the control group ($P = 0.001$) compared with the HT group ($P = 0.017$). There was no difference between the groups in mean sperm concentration and number of round and inflammatory cells. On the first day, there was no difference in sperm viability between groups. There was a significantly higher ($P = 0.001$) percentage of viable sperm in the HT group (86.8 $\pm$ 5.6) compared with the control group (48.8 $\pm$ 21.8) on day 28.**

CONCLUSIONS: **SCI increased the number of round and inflammatory cells and diminished sperm viability in both groups. HT promoted greater sperm viability in rats with SCI.**

INTRODUCTION

Spinal cord injury (SCI) has an estimated incidence of 20–50 cases per 1 million per year and is associated with major clinical, social, and economic issues. Almost half of all injuries occur between the ages of 16 and 30 years, and 80% of victims are male. Car accidents, falls, and gunshot wounds are the leading causes.[1-4] SCI is a debilitating clinical condition not only because of motor and sensory loss but also because of its chronic medical complications, such as coronary artery disease,[5,6] bladder dysfunction,[7] urinary tract infections,[8] and infertility.[9,10]

Male patients with SCI are often at the peak of their reproductive life and had intended to father children[9] but now have to deal with infertility. The quality of the sperm collected in men with SCI is poor (millions of sperm are dead and/or immotile), and the number of leukocytes in the semen is huge.[10] Semen analysis parameters of men with SCI are unique for this population regardless of the method of retrieval, generally demonstrating normal sperm concentration but abnormally low sperm motility and viability. A recent study showed that administration of an oral agent (probenecid) known to interfere with the pannexin 1 cellular membrane channel improved sperm motility in men with SCI.[11] Therefore, studies investigating semen quality in patients with SCI are important for successful fertilization following assisted reproductive techniques. Hyperbaric therapy (HT) may be used in patients with SCI to reduce hypoxia of

Key words
- Hyperbaric
- Infertility
- Oxygen therapy
- Rats
- Semen analysis
- Spinal cord injury

Abbreviations and Acronyms
HT: Hyperbaric therapy
ROS: Reactive oxygen species
SCI: Spinal cord injury
WBC: White blood cell

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damaged tissues, decrease local inflammation, and reduce the degree of secondary lesion, which is related to enzyme release, acute inflammation cascade, and neuronal tissue ischemia.[12] The goal of this study was to evaluate the effectiveness of HT treatment on sperm quality in a rat model of SCI and correlate these findings with histologic analysis of the testicles.

MATERIALS AND METHODS

Sample Size and Randomization

The sample size was calculated by PASS software (NCSS Statistical Software, LLC, Kaysville, Utah, USA), with a confidence interval of 95%, sampling power of 80%, and mean ± SD of sperm viability of 78.9 ± 5.4 to reach a statistical significance of $P < 0.05$.[13] The study subjects were 18 male Wistar rats 10–12 weeks of age (equivalent to adults) and weighing 250–300 g. The rats were randomly distributed to either the HT group ($n = 9$) or the control group ($n = 9$) (Figure 1). Animals manifesting cardiac arrest, urinary tract infections, and respiratory insufficiency were excluded. The randomization was performed using Microsoft Excel (Microsoft Corp., Redmond, Washington, USA). Rats were randomly assigned number 1 (for control group) or 2 (for intervention group).

Study Design

On the first day, 18 Wistar rats were submitted to surgery. The first biopsies were performed on the left testicle before SCI so that the samples were not influenced by the surgical spinal trauma. The second biopsy was performed on the right testicle on the 28th day. Testis biopsies were performed on different sides so that the previous biopsy procedure would not interfere with the results.

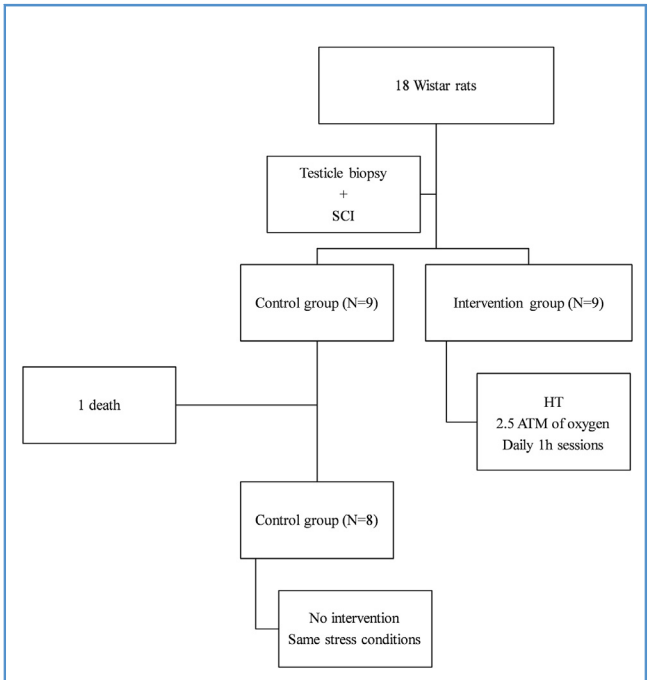

Figure 1. Flowchart of rats submitted to hyperbaric therapy (intervention group) or assigned to control group. SCI, spinal cord injury; HT, hyperbaric therapy.

The intervention group ($n = 9$) had daily 1-hour sessions at 2.5 atm oxygen for 7 days; the first HT session was performed 12 hours after SCI. The parameters of HT were defined in a previous study by our group in rats with SCI.[14] The control group ($n = 9$) was submitted to a similar process inside the hyperbaric chamber, but the pressure was turned off. In a previous study evaluating an experimental model of cryptorchidism in rats, histologic features of testicular damage were detected in the testes submitted to cryptorchidism for the 15-day period as well as the 30-day period.[15] Therefore, we chose to perform testicular biopsy 30 days after the surgical spinal trauma.

All rats were euthanized on the 28th day. Motor assessment of the rats was performed on days 1, 3, 7, 14, 21, and 28. The motor evaluation was measured by the Basso, Beattie, and Bresnahan scale.[16] Figure 2 shows a timeline of our study.

Animal Care

Treatment of all animals was in accordance with the "Experimental Model of SCI: Management Guidelines"[17] and approved by the Animal Ethical Committee. All rats were housed individually in cages and kept in a specific room with a 12-hour light-dark cycle (light from 7 AM to 7 PM). Temperature and air humidity were maintained at 23°–26°C and 62%–68%, respectively. The animals were supplied with water and food ad libitum.

The animals were euthanized after 4 weeks in accordance with the requirements of the ethics committee on animal research. After the animals were euthanized, they were discarded in milky white bags and kept in a freezer at −20°C. They were collected by a specialized service for disposal of biologic material and incinerated.

Surgical Technique of SCI

Antibiotic prophylaxis was instituted 5 days before and 5 days after the surgical procedure and consisted of 320 mg of amoxicillin and clavulanic acid diluted in 500 mL of water for each rat. On the day of the surgical procedure, the rats were anesthetized with xylazine (50 mg/kg) and ketamine (50 mg/kg). Ceftriaxone (100 mg/kg) was used for intraperitoneal antibiotic prophylaxis. The skin was incised, and the connective and muscle tissue were bluntly dissected to expose T8 to T10 vertebral laminas. The animals were then submitted to T9-T10 laminectomy under a surgical microscope view (DF Vasconcelos, Valença, Rio de Janeiro, Brazil). After the spinal cord was exposed, SCI was reproduced by a 25-g weight drop from a height of 12.5 mm (mild lesion) using the MASCIS NYU (New Jersey, USA) (Figure 3). Postoperative analgesia consisted of tramadol (1.5 mg/kg) in the first 48 hours after the procedure. Hygiene care was provided as well as maneuvers to empty the bladder and evaluate possible signs of infection.

HT Protocol

HT was performed in a hyperbaric chamber (Janus & Pergher, Porto Alegre, Rio Grande do Sul, Brazil) (Figure 3). The animals in the intervention group ($n = 9$) were submitted to 1-hour sessions of HT at 2.5 atm oxygen, daily, for 7 days, with the first session initiated 12 hours after SCI. The intervention group ($n = 9$) was