Hypertonic Saline as a Treatment for Acute Spinal Cord Injury: Effects on Somatic and Autonomic Outcomes as Observed in a Mouse Model

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In the United States, there are approximately 200,000 to 400,000 patients with spinal cord injury (SCI), with an estimated incidence of 1 in 1000 people per year.1,21 Because the people involved are often young, between the ages of 16 and 25 years, the amount of lost productivity to society is significant.21 As such, there have been many initiatives and approaches to understanding and treating SCI.21,28

Claire Hulsebosch, in her 2002 review paper, summarizes 11 prime targets for intervention in SCI.21 She lists: 1) reduction of edema and free-radical production, 2) rescue of at-risk neural tissue, 3) control of inflammation, 4) rescue of at-risk neurons/glial cells, 5) repair of demyelination, 6) promotion of neurite growth, 7) cell replacement, 8) gap bridging or transplantation, 9) retraining or relearning motor tasks, 10) restoration of function through electrical stimulation, and 11) relief of chronic pain.

There has been a great effort to address these targets through a variety of interventions. Methylprednisolone as a treatment has been used clinically in humans, but remains somewhat controversial in its outcome.4,14,48 Other techniques have also been introduced, including various therapeutics to modulate immune function,18,24 nutritional supplementation,20,38 cell transplantation,11,41 tissue engineering or guided tissue regeneration,5,45 and physical therapy.12,23,32 Many of these techniques have reported some measure of success, but no one method or one combination of method has emerged as the clear treatment for SCI.

Previous work in our laboratory has suggested that hypertonic saline (HTS) may be an effective intervention in SCI. Previous work with rat ischemic injury models in our laboratory revealed that kinematic scores (lower extremity [LE] motion, and Basso, Beattie, and Bresnahan [BBB] scales) were improved in treated groups.30,42–44,47,50 In this study, HTS is administered to mice receiving a severe low thoracic spinal cord contusion. Mice were assessed for both autonomic outcomes (bladder function) and somatic outcomes (Modified BBB, Basso Mouse Scale [BMS], and LE motion) compared with nontreated and equivalent-volume saline treatment.

The somatic (BBB, BMS) scores were most improved in the 24-hour treatment group. The autonomic (bladder) outcomes were improved in all mice receiving treatment. Mice receiving HTS were more likely to recover bladder function compared with the control group. Administration of HTS did not consistently result in improved lower extremity function, but no mouse seemed to have fared worse than mice receiving no treatment.

MATERIALS AND METHODS

Spinal Cord Injury

All procedures and protocols have been approved by the Temple University Institutional Animal Care and Use Committee. The animals used were female C57Bl/6 mice (Taconic Inc., Hudson, NY) weighing approximately 15 to 20 g. Before surgery, the mice were housed for 7 days for acclimation and observation. At all times, the mice were allowed free access to food and water. Mice were exposed to a light/dark cycle approximating 12/12 hours. The animals were anesthetized with Avertin (1 g 2,2,2-tribromoethanol dissolved in 0.62 ml isoamyl alcohol and 79 ml micropore-filtered water at 40°C) injected at 0.1 ml/5 g into the peritoneum. The body temperature during all surgical procedures and during recovery from anesthesia was maintained via a heat lamp and/or heating pad. The depth of anesthesia was monitored via withdrawal response and respiratory rate.

After anesthetic induction, the animals had their bladders emptied via a Credé maneuver. After emptying, the animals’ backs and preauricular areas were shaved. The eyes were covered with a layer of ophthalmic petroleum-based ointment. The animal was placed prone on a heating pad. The surgical site was prepared using Betadine. The surgery was performed under rigid aseptic conditions. A dorsal thoracic incision was performed, followed by blunt dissection of the
dorsal fat pad. Care was taken to identify the fat pad vessel exiting at T4–T5. Tissue dissection was performed to expose the laminae, centering at levels T8–T10. Using microscissors, the ligamentum flavae were sectioned and the laminae were cut. The laminae were reflected to expose the dorsum of the dura mater of the spinal cord. The animal was transferred gently to the modified Adson’s forceps holder provided with the Infinite Horizons Impactor device (IH Impactor; PSI Inc., Lexington, KY). The Adson’s forceps were fitted snugly on the lateral spinal column cranially and caudally to the laminectomy site. The dura was inspected and positioned level underneath the impactor. The impact force was chosen to be 60 kdyne to provide a moderate to severe injury. A sample injury is shown (Fig. 35.1). The actual force, depth of compression, and the duration are measured with the IH device. These parameters were consistent with previously published work on mouse SCI models.12,22,29 On the first day after injury, all of the mice were routinely assessed. Mice with a total BMS score of at most 1 on either leg or a total BMS score of at most 2 were deemed to have received a clinically significant injury. Mice with a modified BBB score of at most 2 on either leg or a total BBB score of at most 4 were deemed to have received a clinically significant injury. Animals that had scores outside these parameters were considered insufficiently impacted and were excluded from the study.

**Hypertonic Solution**

The facial vein was identified and cannulated temporarily for intravenous injection. HTS (7.5%, 5 ml/kg) was used as the therapeutic agent. HTS was prepared under sterile conditions by the pharmacy at Temple University Hospital (80 ml of 23.4% HTS mixed with 170 ml sterile water). Once the vein was entered, the solution was slowly injected. The facial incision was closed with skin staples after injection.

**Behavioral Assessment**

Animals were assessed on postoperative days 1, 7, 14, 21, and 28. Animals were simultaneously assessed for BMS and Modified BBB10 scores according to published protocols. Animals were also evaluated daily for spontaneous LE motion. The first postoperative day of spontaneous LE motion was recorded.

**Bladder Assessment**

Mouse bladder function was assessed twice daily. All injured mice initially required twice-daily Credé maneuvers (manual bladder emptying). The emptied urine was weighed and followed as a total daily mass of expressed urine in milligrams. Using volumes described in the literature,36,44 we determined less than 500 mg/d urine expressed as a value to declare a bladder potentially recovered. Animals who produced daily totals of less than 500 mg for 3 consecutive days were considered to have recovered a functional bladder (NB: this translates to six consecutive bladder emptyings and measurements). The animals that were considered to have recovered bladder function continue to have their bladders palpated twice daily, and were assigned sizes corresponding to measured masses. With practice, a handler could accurately estimate bladder volume (small, ≅ 200 mg; medium, ≅ 300 mg; or large, ≥ 400 mg). Animals with large bladders for three consecutive measurements (1.5 d) were considered as having been erroneously assigned a functional bladder or possibly having lost a functional bladder and were returned to twice-daily Credé maneuver emptying. Combined with unpublished data, this event occurred only once in more than 50 examined mice.

**Experimental Design and Statistical Analysis**

The design was a prospective nonblinded study. Animals were divided into groups: no treatment (9 animals), treatment with 7.5% HTS at 3 hours after injury (4 animals), treatment with 7.5% HTS at 8 hours after injury (4 animals), and treatment with 7.5% HTS at 24 hours after injury (4 animals). Animals were assessed to 28 days and then killed. Animals that had injuries to their lower extremities (e.g., flank wound or toe infection) were excluded.

On postoperative days 1, 7, 14, 21, and 28, animals were tested for kinematics. The BMS scale and the BBB scale (modified for mice10) were used.

Bladders were emptied by the Credé maneuver twice daily. The drained urine was measured by weight. Animals that had less than 500 mg daily of expressed urine for 3 concurrent days were considered to have “passed” the bladder protocol and were considered to have the ability to spontaneously drain their bladder. The first day of the 3 concurrent days was then considered the day of recovery. All animals passing the bladder protocol continued to have their bladders gently palpated twice daily to be certain that no animal would have unchecked urinary

**FIGURE 35.1.** Sample injury. The mouse has been injured using a force of 60 kdynes. The specimen has been dissected out from the mouse.
retention. A mouse found to have a large bladder (400 mg by palpation estimation) for three consecutive palpations was considered to have been erroneously passed and was returned to the regular bladder draining protocols.

Outcomes were analyzed as follows. Kinematic scales (BMS, BMS) were analyzed with a mixed model analysis of covariance (ANCOVA) for repeated measures on rank. Bladder recovery days were analyzed via a t test, two-sample assuming unequal variances. LE first observed motion was analyzed using an accelerated failure-time model.

RESULTS
Kinematic Testing
Kinematic testing consisted of BBB, BMS, BMS subscore, and LE motion. One treatment group (HTS at 24 hours) seemed to have a more prominent improvement compared with the other treatment groups and the nontreated groups (Fig. 35.2). Statistical analysis revealed that animals treated at either 3 hours, 8 hours, or 24 hours were statistically improved versus control at several time points (ANCOVA; \( P < 0.05 \)). The modified BBB scale was more likely to identify statistically significant points than the BMS scale. For animals treated with HTS at 3 hours \((n=4)\), significance occurred at Days 1, 7, 14, 21, 28 according to the BBB scale, and on Day 14 according to the BMS scale. For animals treated with HTS at 8 hours \((n=4)\), significance occurred at Days 14, 21, and 28 according to the BBB scale, and at 28 days according to the BMS scale. For animals treated with HTS at 24 hours \((n=4)\), significance occurred at days 14, 21, and 28 according to the BBB scale, and at 14 and 28 days according to the BMS scale.

For the first observed LE motion, all animals recovered in similar time intervals, with first extremity motion appear-

![FIGURE 35.2. A, BBB results for the first study group reveal improvement with HTS. Significance is reached at Days 14, 21, and 28 as compared with nontreatment. B, BMS results for the first study group reveal improvement with HTS. Significance is reached at Days 14 and 28.](image-url)
ing between 2 and 4 days (Fig. 35.3). It is satisfying to note that the force of the impact did significantly correlate to the interval for leg recovery.

**Autonomic Testing (Bladder Functional Recovery)**

Bladder functional recovery was significantly improved by treatment with HTS (Fig. 35.4). For example, all treated groups improved more significantly than the nontreated group. This reached statistical significance for each group compared with the untreated group ($P < 0.05$).

**DISCUSSION**

The impact of SCI on the individual and society cannot be underestimated. Approximately 200,000 to 400,000 Americans deal with SCI on a chronic basis. The community of people with SCI has needs that vary according to their injury level. One way to help this community is to understand the mechanisms that underlie SCI and to be able to develop treatment strategies tailored to the components of SCI.

One can consider the components of SCI in terms of primary injury (direct energy transfer from mechanical or chemical injury) and secondary injury, deriving from vascular changes (e.g., microvascular thrombosis), necrosis, cellular invasion, oxygen-radical formation, and neurotoxicity. Certainly, all of these elements contribute to the grey and white matter destruction of the spinal cord after injury. Prevention (e.g., seatbelts, driving laws, diving laws, etc.) may help prevent primary injury, but it is in the arena of secondary injury that the injury pathways may be treated to preserve function in the injured individual.

The cellular mechanisms of secondary injury revolve around the migration of leucocytes and other inflammatory cells to the site of injury. There is controversy regarding the relative role of the invading cells, with some scientific groups suggesting that the invading cells are more detrimental than helpful, and other groups thinking that certain invading cells are helpful. There are experimental therapies designed at introducing activated macrophages at the site of SCI to treat SCI in humans. Many scientists think that immune modulation after injury ameliorates the injury. What is less controversial is that inflammatory cells do arrive to an area of SCI in a relatively ordered fashion. By 8 to 24 hours after injury, leukocytes are present.

HTS is a deceivingly simple substance. Essentially, it is sodium chloride and water mixed to provide a concentration of sodium chloride greater than that of average human plasma. This simple substance, however, has received much attention in the trauma literature as a compound useful for fluid replacement in traumatic injury, being easy to manufacture, carry, store, and administer.

HTS is frequently used in trauma situations, having been found to improve tissue oxygenation and perfusion. In animal models, improvement is noted in outcome measures such as oxygenation and perfusion indices, tumor necrosis factor-α concentrations, interleukin-6 concentrations, intercellular adhesion molecule (ICAM)-1 expression, pulmonary perivascular edema, polymorphonuclear cell (PMN) sequestration, and mortality when compared with isotonic saline administration. Additionally, HTS is known to reduce endothelial cell swelling and to promote reperfusion.

However, HTS apparently functions as more than just a simple volume-replacement substance. Many different researchers have investigated the role that HTS has in affecting the performance of neutrophils, cells known to participate intimately in the cascade of tissue damage.

Angle et al. studied neutrophil adhesion molecule expression in hemorrhagic shock and neutrophil-mediated organ injury. In both a mouse model and in human volun-

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*FIGURE 35.3. LE first observed motion. All mice recover leg motion between 2 and 4 days. Error bars represent standard deviations. Tx, treatment.*

*FIGURE 35.4. Bladder functional recovery. Mice improved with treatment with HTS. The percentage of mice that recovered bladder function is shown. When compared with no treatment, each group improves with statistical significance ($P < 0.05$). Bars reflect the standard deviation.*
nants, HTS was found to depress neutrophil L-selectin expression as well as the β2-integrin, CD11b, thereby reducing the neutrophil ability to perform phagocytosis.

In a series of investigations by Ciesla et al.,6,7 the effect of HTS on neutrophils was studied. HTS attenuates the neutrophil p38 mitogen-activated protein kinase signaling pathway, thereby reducing the PMN cytotoxic response. CD11b/CD18 interaction with endothelial ICAM-1, essential for PMN tissue damage, was also assessed. They note that the timing of exposure to HTS is critical. PMNs exposed to HTS before priming (with platelet-activating factor) reduced CD11b/CD18 expression, whereas PMNs exposed to HTS after priming increased CD11b/CD18 expression. Work by Junger et al.25 also concludes that HTS administration administered before priming, suppresses PMN functions. In a series of investigations by Rizoli et al.,39,40 HTS is shown to lessen lung neutrophil sequestration, to prevent CD11b up-regulation, and to reduce surface L-selectin expression. Work by Deitch et al.9 with HTS resuscitation in rats also revealed that CD11b and CD18 expression were increased in rats resuscitated with Ringer’s lactate, but not with 7.5% HTS. Thiel et al.46 note that HTS reduced up-regulation of β2-integrins and decreased L-selectin shedding, although they suggest the effect may be from the sodium cation rather than just hyper-tonicity.

Using knock-out mice, Farooque et al.13 demonstrated that mice lacking ICAM-1 and P-selectin had improved motor function outcomes compared with wild-type mice. Work by Weaver et al.48 and Gris et al.17 explores the effect of introducing antibodies to directly inhibit the CD11d/CD18 complex. In a clip compression model, this group demonstrated improved outcomes with respect to sensory, autonomic, and motor function.

From previous experimentation in a rat spinal cord compression/ischemia model (30, 42–44, 47, 50), we noted improvements in mobility and motion in animals treated with HTS. In our laboratory, work by Spera et al.42 on leukocyte adhesion in rats treated with HTS found that leukocyte adhesion, as measured by shear and rolling, was reduced in animals treated with 7.5% HTS. In a follow-up study, Spera demonstrated increased blood flow to rat spinal cords treated with HTS.43 Rats treated with HTS also demonstrated functional outcome benefits. For example, rats receiving HTS 1 minute after compression revealed earlier times to bladder voiding, earlier times to LE motion, and superior BBB outcome score compared with rats receiving either no treatment or equivalent volumes of lactated Ringer’s solution.44

Support for the notion that cellular invasion may be critical in secondary injury comes from other models as well. In a rabbit model of SCI caused by ischemia, drugs administered to inhibit mononuclear phagocytes (chloroquine and colchicine) resulted in improved motor outcome.16 In work by Weaver et al.49 and Gris et al.17 antibodies to CD11d receptors have also shown promise in improving autonomic (autonomic dysreflexia) and somatic (BBB scores) outcomes,17,49 further supporting the therapeutic role for modulating the immune response in SCI.

Because HTS has been shown to directly affect the regulation of surface receptors critical for neutrophil migration to the site of injury, and because HTS directly effected leukocyte rolling and adhesion, and because direct inhibition of these same receptors by other mechanisms (knock-out mice and antibodies) improved outcome, we hypothesized that HTS would be an effective treatment for SCI on the basis of its ability to reduce neutrophil migration.

Compared with controls, the trend was for treated mice to have improved BBB and BMS scores (Fig. 35.2). Statistical significance was reached at several points (see above), but, most notably, the 24-hour treatment group had the most favorable trend and the most points of significant improvement.

Compared with controls, mice in each treatment group were more likely to recover bladder function (Fig. 35.4). Using a t test, two-samples assuming unequal variances, animals treated at 3, 8, and 24 hours all were significantly improved versus the untreated control (P = 0.001, 0.009, and 0.001, respectively).

Treatment with HTS resulted in a greater likelihood of ambulatory and bladder recovery. There may be an aspect of volume restoration that may be protective for SCI. Given the role for the bulbospinal tract to coordinate the detrusor muscle and the external urethral sphincter to permit murine urination,31,36 it may be that increased preservation of these descending tracts by HTS administration at a time coincident with leukocyte migration explains the improvement in bladder function in the same way that preservation of corticospinal tract preservation would aid in ambulatory function. It is known that only approximately 5% of the descending tracts need to be preserved to allow for a measure of functionality in the injured spinal cord.15

The measure of the first day of LE motion did not prove significant to discriminate between different groups. It is interesting to note, however, that the force of the impact, as measured by the IH impactor transducer, did significantly correlate with the time to lower extremity recovery. This result is intuitively satisfying and lends support to the impact contusion model as a mechanism to generate SCI.

In summary, HTS treatment after SCI was found to result in improved ambulatory ability with later treatment (at 24 hours) and improved bladder recovery with HTS at each treatment time from 3 to 24 hours. The treatment time chosen, of 3 to 24 hours after injury, reflects a reasonable treatment window in clinical practice. This suggests that HTS administration could be a clinically relevant treatment for SCI suitable for future clinical trials. Future work is planned to
further investigate the role of HTS on modulation of neutrophil invasion in the mouse model of SCI.

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REFERENCES


