A Preliminary Study of Intravenous Surfactants in Paraplegic Dogs: Polymer Therapy in Canine Clinical SCI

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ABSTRACT

Hydrophilic polymers, both surfactants and triblock polymers, are known to seal defects in cell membranes. In previous experiments using laboratory animals, we have exploited this capability using polyethylene glycol (PEG) to repair spinal axons after severe, standardized spinal cord injury (SCI) in guinea pigs. Similar studies were conducted using a related co-polymer Poloxamer 188 (P 188). Here we carried out initial investigations of an intravenous application of PEG or P 188 (3500 Daltons, 30% w/w in saline; 2 mL/kg I.V. and 2 mL/kg body weight or 300 mL P 188 per kg, respectively) to neurologically complete cases of paraplegia in dogs. Our aim was to first determine if this is a clinically safe procedure in cases of severe naturally occurring SCI in dogs. Secondarily, we wanted to obtain preliminary evidence if this therapy could be of clinical benefit when compared to a larger number of similar, but historical, control cases. Strict entry criteria permitted recruitment of only neurologically complete paraplegic dogs into this study. Animals were treated by a combination of conventional and experimental techniques within ~72 h of admission for spinal trauma secondary to acute, explosive disk herniation. Outcome measures consisted of measurements of voluntary ambulation, deep and superficial pain perception, conscious proprioception in hindlimbs, and evoked potentials (somatosensory evoked potentials [SSEP]). We determined that polymer injection is a safe adjunct to the conventional management of severe neurological injury in dogs. We did not observe any unacceptable clinical response to polymer injection; there were no deaths, nor any other problem arising from, or associated with, the procedures. Outcome measures over the 6–8-week trial were improved by polymer injection when compared to historical cases. This recovery was unexpectedly rapid compared to these comparator groups. The results of this pilot trial provides evidence consistent with the notion that the injection of inorganic polymers in acute neurotrauma may be a simple and useful intervention during the acute phase of the injury.

Key words: canine paraplegia; P 188; paraplegia; polyethylene glycol; polymer; spinal cord injury; surfactant

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INTRODUCTION

A development arising from the chemical engineering of membranes was critical to the development of monoclonal antibodies—the ability to fuse two different cells into one. In particular, the use of the hydrophilic polymer polyethylene glycol (PEG) to fuse mouse myeloma cells with lymphocyte (so-called hybridomas) permitted the establishment of immortal cell lines producing antibodies to specific cellular components. More recently, this same cell fusion technique is showing promise to revolutionize another scientific discipline, the medical management of soft tissue trauma.

We have been investigating the use of PEG to reunite and fuse transected cell processes, and to seal anatomical breaches in cell membranes produced by mechanical insult (Borgens, 2001). In particular, we have shown that a brief topical application of PEG can anatomically and physiologically fuse severed axons in the white matter of adult guinea pig spinal cord (Shi et al., 1999), or immediately repair crushed axons restoring physiological functioning in isolated spinal cords (Shi and Borgens, 1999). A related co-polymer, P 188 (Marks et al., 2001) has also been shown to seal membranes in a variety of cell injury models ranging from electric shock induced myonecrosis (Lee et al., 1992a,b) to a testicular reperfusion injury model in rats (Palmer et al., 1998; Borgens, 2003). Administration of PEG, as well as P 188 (Borgens et al., 2004) through the vasculature was just as effective as a topical application to the exposed spinal cord. The purpose of this preliminary clinical study was to determine the safety and possible benefit of intravenous polymer administration in treating naturally occurring neurologically complete paraplegia in dogs.

Clinical paraplegia in dogs has been a useful model for the development of emergent human clinical therapies for spinal cord trauma. Such veterinary trials preceded the first tests of 4-aminopyridine and oscillating field stimulation in human SCI (Borgens et al., 1993, 1999; Blight et al., 1991) as a means to further evaluate possible therapies for SCI prior to clinical tests in human beings (Hansebout et al., 1993; Hayes et al., 1993; Shapiro et al., 2004). Here we provide preliminary results of intravenous injection of PEG or P 188 under such clinical conditions.

MATERIALS AND METHODS

Overview: Admission and Treatment

Dogs were included in the study if they presented at the clinic with severe, acute, neurologically complete paraplegia. These animals were between 2 and 8 years of age, weighing 40 lb or less, with T3–L3 myelopathy (upper motor neuron signs) resulting from acute intervertebral disk herniation. They were required to have been paraplegic (i.e., absence of locomotion, conscious proprioception, deep and superficial pain perception) for 72 hours or less when brought to the Veterinary Medicine Teaching Hospital (VMTH). Dogs satisfying these criteria were admitted to the emergency services of the VMTH at Texas A&M University, College Station, Texas, and at Purdue University, West Lafayette, Indiana. An identical protocol for admission, neurological evaluation, treatment and follow-up was adhered to by each research center. All procedures were approved by the Animal Care and Use Committee at both Universities. During the initial clinical evaluation owners were asked to review a document explaining the experimental treatment and then requested to sign an informed consent should they wish to participate in the study.

Usually within 1/2–2 h of admission, each dog received a diagnostic imaging examination (Fig. 1), and a videotaped neurological examination that evaluated the hindlimbs for (1) superficial and deep pain, (2) conscious proprioception (hindlimb placement), (3) load-bearing and voluntary locomotion, and (4) segmental spinal reflex testing (patellar, flexor withdrawal, cranial, tibial and gastrocnemius reflexes). Results of the neurological examination were also used as functional measures of outcome and were quantitatively scored using previously reported techniques and methods (Borgens et al., 1993, 1999) briefly described below. All dogs admitted to the study had severe clinical signs of compressive SCI, secondary to severe acute disk herniation. This clinical condition was characterized by complete paraplegia, urinary and fecal incontinence, lack of deep and superficial pain response, and the complete absence of evoked potential conduction through the injured segment of spinal cord as measured by SSEP in those dogs tested. This condition represents the worst clinical grade, defining paraplegic
dogs with the worst possible prognosis (so-called grade 5 lesions; Coates, 2000). These tests and others were also used as exclusion criterion so that dogs with neurologically incomplete injuries were not included in the trial.

A complete blood count, blood chemistry, and urinalysis were submitted from each dog. Survey radiographs and myelography were performed under general anesthesia. In some cases, computed tomography (CT) imaging was available at the Texas center, while electrophysiological study of nerve impulse conduction through the lesion by evoked potential testing (Somatosensory Evoked Potentials or SSEP) was performed routinely at Purdue University. Twelve of a total of 19 PEG–treated dogs and 6 of 16 P 188–treated dogs were treated and evaluated at Purdue University, the balance at Texas A&M University. In all cases, rapid surgical management of spinal trauma was emphasized. Dogs routinely were moved to surgery after the radiological examination within the day of admission.

All dogs received an initial intravenous injection of either PEG (~3500 Da, Sigma Chemical Company, St. Louis, MO; #P2906: 30% w/w in sterile saline; 2 mL/kg administered over 15 min) or P 188 (Flocor; 150 mg P 188/mL, 2 mL/kg body weight) usually while in radiology. A second intravenous injection of PEG (or P 188) was administered usually within 4–6 h of the initial injection. This molecular weight (MW) and concentration was initially selected on the basis of guinea pig trials (Borgens and Shi, 2000; Borgens and Bohnert, 2001; Borgens et al., 2002), and later preliminary experimentation in dogs where a slightly higher MW and lower percent solution (reduced from 50% to 30%) provided an ef-
effective solution for intravenous injection of reduced viscosity. Sterile injectable P 188 was provided by Cytrex Corporation. Following confirmation of a focal, extradural compressive spinal cord lesion within the T₃–L₃ region of the spinal cord, the dogs received preoperative injection of methylprednisolone sodium succinate (MPSS; 30 mg/kg body weight I.V.), and then underwent general anesthesia and decompressive surgery. The dogs remained hospitalized and were monitored for 7–10 days. The neurological examination was repeated approximately 3 days (74 ± 9 h) post-surgery, 1 week post-surgery at discharge (6.8 ± 1.2 days), and at 6–8 weeks post-surgery at recheck. An identical procedure was followed using injections of P 188 (2 mL/kg body weight or 300 mL P 188 per kg).

**Neurological Examination**

We have used an identical neurological examination provided in detail in previous reports (Borgens et al., 1993, 1999) and provide only a brief summary here. The dog was placed in lateral recumbency and tested for the presence of superficial pain, deep pain, and supported upright for tests of conscious proprioception. Skin on the flank and limbs was pinched sharply with a hemostat during tests for superficial pain responses. Deep pain response was determined by a forceful squeezing of the medial and lateral digits of the paw. Positive responses were provided for comparison by testing the forelimbs. Voluntary ambulation was scored by observing the animal’s gait and their ability to climb stairs. A score of 1–5 (Borgens et al., 1993, 1999) quantified each four individual components of the neurological evaluations. Briefly, for superficial and deep pain, 1 = no detectable response; 2 = a response at the limits of detection, indicated by an increased state of arousal, increased respiration or pulse; 3 = consistent attention to the painful stimulus but without any overt defensive behavior; 4 = mildly defensive behavior such as abrupt turning of the head towards the stimulus, and whining; 5 = completely normal response to painful stimuli including yelping, biting, and aggressive behavior. Scores within the range of 1–5 were obtained for both sides of the body and divided by two. In cases where dogs were of a stoic demeanor, and/or when the reaction to pain was at the limit of detection, the responses of the dogs eyes (at the exact time the painful stimuli was applied) were filmed and later studied to determine if a score greater than 1 should be assigned. Conscious proprioception (CP) and weight support were tested in a supported upright position. For testing CP, a hind paw was turned “under” so that the dorsal surface of the paw (and the animal’s weight) rested on the table. A normal animal briskly re-places the paw to a normal stance. However, paraplegic animals remain in this “knuckled under” posture for extended periods. Testing the foreleg provided a positive control. The test was performed on each side of the body and scored on each side: 1 point = complete absence of CP, and 2.5 points for a positive CP response. These scores were then summed for each animal. Voluntary locomotion was also evaluated with a 1–5-point scoring system; 1 = complete inability to step or ambulate; 2 = stepping at the limit of detection, at best a few steps before falling; 3 = longer sequences of stepping, poorly coordinated before falling, but unable to climb stairs; 4 = more robust and effective walking but with clear deficits in coordination, effective weight support, and able to climb stairs; 5 = completely normal voluntary walking, indistinguishable from a normal animal. All neurological exams were videotaped for reference and half point scores were permitted at the examiner’s discretion. The individual group scores for each of these latter 4 outcome measures were available for comparison (see below), and the scores obtained from all four tests were summed to provide a Total Neurological Score (TNS) for each animal. Thus, the range of a possible TNS for any one dog was 4 (a totally paraplegic animal) to 20 (a totally normal animal, indistinguishable from an uninjured one).

**SSEP Testing**

Testing was carried out on sedated dogs. Bipolar stimulating needle electrodes were inserted subcutaneously in the hind limb at the distal popliteal area approximately 0.5–1 cm apart to stimulate the tibial nerve. A similar procedure was used to stimulate the median nerve of the forelimb. (This latter procedure insured that activation of the peripheral nerve of the limb resulted in a measurable evoked potential at the cortex as this neural circuit is above the level of the spinal cord injury and unaffected by it.) Trains of square wave stimulations (0.5–3.0 mA amplitude, 200/min) were applied to evoke compound nerve impulses from these nerves. To record ascending evoked potentials, scalp needle electrodes were inserted subcutaneously over the somatosensory cortex contralateral to the side of the dog stimulated, while reference electrodes were inserted on the opposite side between the mastoid and the pinna of the ear. Stimulation, recording, and the management of data was carried out using a Nihon Kohden ME# B–5304K Neuropak 4. Effective placement of recording electrodes was facilitated by stimulation of the median nerve at the outset of each testing period. This procedure provided a positive control recording to validate the inability to record evoked potentials stimulated at the hind limb, but whose ascending potentials were blocked by the spinal cord lesion. Further methodological details can be found in Borgens et al. (1999).
Anesthesia and Decompressive Surgery

General anesthesia was begun with a subcutaneous injection of atropine (0.5 mg/kg) or glycopyrrolate (0.01 mg/kg) and oxymorphone (0.05 mg/kg) or hydromorphone (0.1 mg/kg), subsequent placement of an indwelling catheter in the cephalic vein, and induction of anesthesia by sodium pentothal (15 mg/kg body weight) or propofol (2–4 mg/kg). Anesthesia was maintained following endotracheal intubation and administration of a mixture of oxygen and isoflurane or halothane. A hemilaminectomy was performed and the spinal cord decompressed secondary to removal of extruded disk material (Borgens et al., 1993, 1999). An autogenous fat graft was harvested and placed over the exposed spinal cord before routine wound closure. All animals were taken to the intensive care unit (ICU) for recovery and post-operative monitoring.

Comparator Groups

A medical decision was made not to use a sham-injected control group in this preliminary study. Useful historical control data could be obtained from peer-reviewed and published studies performed at the Purdue Center (Borgens et al., 1993, 1999). All of these dogs were (1) also admitted to veterinary clinical trials restricted to only neurologically complete cases of acute canine paraplegia secondary to acute intervertebral disk extrusion, (2) received identical conventional medical management as described above, (3) were neurologically evaluated by identical methods (Borgens et al., 1993, 1999) and excluded from the studies by identical exclusion criteria (Borgens et al., 1993, 1999), (4) were evaluated at the same time points, and (5) in most cases, their neurological scores were derived by blinded evaluation by most of the same investigators participating in this trial (R.W., G.B., J.T., R.B.). For purposes of comparison, complete medical records, score sheets, and videotapes were available for all 11 sham-treated dogs from 1993 (Borgens et al., 1993) and 13 of 14 control dogs from 1999 (Borgens et al., 1999)—24 dogs total for comparison to 19 PEG–treated dogs and 16 P 188–treated dogs. The only omission was due to a relatively “late” decompressive surgery performed 3 days post-injury in one of two studies (Borgens et al., 1999). In the latter clinical trial, the experimental application (oscillating field stimulation) was delayed in 12 experimental dogs for ~96 h after surgery to determine what, if any, early functional recovery could be associated with surgery and steroid treatment alone. The neurological status of this subset of dogs was reported (Borgens et al., 1999). These data then provided a total of 36 control dogs to compare to 19 PEG–treated dogs and 16 P 188–treated dogs at the 3-day time point, and 24 control dogs for comparison at the 1-week and 6–8-week neurological checkups. Comparisons between mean scores, and between proportions, were made using Student’s t test, two tailed, or the Welch variation, and Fisher’s exact test, respectively, on Instat™ software.

RESULTS

General Responses to Treatment

There were no major complications due to myelography (used in every case to determine the mode of injury), decompressive surgery, or the administration of PEG or P 188. Dogs tolerated the “slow push” injections of the polymer with no obvious change in their clinical status. There were no deaths attributable to any experimental or conventional intervention. There was no evidence of increased wound infection, difficulty in bladder management over that routinely encountered, or rehospitalization for reasons secondary to the conduct of the study. Rather, the clinical condition of study dogs was observed to be unremarkable, and their neurological status improved faster and more completely than expected. Radiology confirmed acute explosive intervertebral disk lesions, typified by severe dorsal displacement of the ventral subarachnoid contrast column and spinal cord or obliteration of the contrast columns because of acute cord swelling from acute, explosive disk herniation.

Early Changes in Neurological Scores

The most sensitive indicator of early functional recovery in clinical cases of neurologically complete canine paraplegia is the return of deep pain in hindlimbs and digits (Borgens et al., 1993, 1999; Coates, 2000). This was evaluated in 17 of the 19 acutely injured PEG-treated dogs approximately 2–3 days after surgery (approximately 24–48 h after the second injection of PEG) and 15 of 16 P 188–treated dogs. Comparison to historical controls at this time point revealed a likely improvement in the early appreciation of deep pain by PEG and P 188 administration. Early in the study, the mean TNS, a number derived largely from recoveries in deep and superficial pain, was unexpectedly elevated compared to the progress of control dogs in past years (Table 1). Various levels of recovery in proprioception, improvement in load bearing in hind limbs, and voluntary walking were observed in 8 of 19 PEG-treated dogs at the 1 week recheck. In the two previous studies, such improvements were not observed in control dogs at this time point (Borgens et al., 1993, 1999). These differences were statistically significant for both PEG and P 188–treated dogs (p = 0.004). An early or later capability to walk was not...
frequently observed in the P 188–treated dogs when compared to PEG-treated dogs.

**Final Recheck**

The TNS of the historical control dogs did reveal modest and progressive improvement by the end of the study period. This mainly resulted from improvements in the quality of pain sensation (Borgens et al., 1993, 1999). This improvement was not matched in any of the other three functional tests. Thirty-seven percent (seven of 19) of the PEG-treated dogs recovered measurable proprioception by 6 weeks and seven of 16 (42%) of the P 188–treated dogs. In the previous study, only one of 24 (4%) of control animals had improved proprioception. Ambulation had recovered in 68% of PEG-treated dogs (13 of 19) and nine of 16 (56%) of P 188-treated dogs by the end of the study (Table 2). This capability compared favorably to only 25% (six of 24) controls that had recovered walking ability by the end of either of the two previous trials.

Qualitatively, the status of polymer injected dogs was very different from historical controls by the end of the

**Table 1. TNS Comparison at All Recheck Periods**

<table>
<thead>
<tr>
<th>Group</th>
<th>N&lt;sup&gt;1&lt;/sup&gt;</th>
<th>3 day</th>
<th>1 week</th>
<th>6–8 weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>?&lt;sup&gt;2&lt;/sup&gt;</td>
<td>SEM&lt;sup&gt;3&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>1. PEG</td>
<td>19</td>
<td>5.1</td>
<td>0.6</td>
<td>19</td>
</tr>
<tr>
<td>2. P 188</td>
<td>16</td>
<td>4.3</td>
<td>0.1</td>
<td>16</td>
</tr>
<tr>
<td>3. Control</td>
<td>36</td>
<td>4.0</td>
<td>0.03</td>
<td>24</td>
</tr>
</tbody>
</table>

**Statistical comparison**

<table>
<thead>
<tr>
<th></th>
<th>1 vs. 3</th>
<th>2 vs. 3</th>
<th>1 vs. 2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.007</td>
<td>0.005</td>
<td>0.002</td>
</tr>
<tr>
<td></td>
<td>0.006</td>
<td>0.03</td>
<td>0.001</td>
</tr>
<tr>
<td></td>
<td>0.3</td>
<td>0.5</td>
<td>0.9</td>
</tr>
</tbody>
</table>

The table provides a comparative data between the experimental and historical control groups at the 6–8-week time points: (1) The total number of dogs evaluated. (2) Mean neurological score. (3) Standard error of the mean. (4) Statistical comparisons between PEG vs. historical cases (top row); P 188 versus historical cases (middle row); and PEG versus P 188 (bottom row) are given for each time point.

**Table 2. Recovery from Spinal Cord Injury at the End of the Study Period**

<table>
<thead>
<tr>
<th>Group</th>
<th>N&lt;sup&gt;1&lt;/sup&gt;</th>
<th>?&lt;sup&gt;2&lt;/sup&gt;</th>
<th>SEM&lt;sup&gt;3&lt;/sup&gt;</th>
<th>Range&lt;sup&gt;4&lt;/sup&gt;</th>
<th>P-Value&lt;sup&gt;5&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Superficial pain</td>
<td>PEG 19</td>
<td>2.2</td>
<td>0.4</td>
<td>1–5</td>
<td>0.03</td>
</tr>
<tr>
<td>P 188 16</td>
<td>3.2</td>
<td>0.39</td>
<td>1–5</td>
<td>0.03</td>
<td></td>
</tr>
<tr>
<td>Control 24</td>
<td>24</td>
<td>1.3</td>
<td>0.1</td>
<td>1–5</td>
<td>0.03</td>
</tr>
<tr>
<td>Deep pain</td>
<td>PEG 19</td>
<td>2.9</td>
<td>0.4</td>
<td>1–5</td>
<td>0.02</td>
</tr>
<tr>
<td>P 188 16</td>
<td>3.1</td>
<td>0.37</td>
<td>1–5</td>
<td>0.02</td>
<td></td>
</tr>
<tr>
<td>Control 24</td>
<td>24</td>
<td>1.8</td>
<td>0.3</td>
<td>1–5</td>
<td>0.02</td>
</tr>
<tr>
<td>Proprioceptive placing</td>
<td>PEG 19</td>
<td>1.7</td>
<td>0.3</td>
<td>1–5</td>
<td>0.03</td>
</tr>
<tr>
<td>P 188 16</td>
<td>2.3</td>
<td>0.3</td>
<td>1–5</td>
<td>0.03</td>
<td></td>
</tr>
<tr>
<td>Control 24</td>
<td>24</td>
<td>1.1</td>
<td>0.08</td>
<td>1–3</td>
<td>0.03</td>
</tr>
<tr>
<td>Ambulation</td>
<td>PEG 19</td>
<td>2.3</td>
<td>0.3</td>
<td>1–5</td>
<td>0.001</td>
</tr>
<tr>
<td>P 188 16</td>
<td>2.2</td>
<td>0.32</td>
<td>1–4</td>
<td>0.001</td>
<td></td>
</tr>
<tr>
<td>Control 24</td>
<td>24</td>
<td>1.3</td>
<td>0.1</td>
<td>1–2.5</td>
<td>0.001</td>
</tr>
</tbody>
</table>

The table provides comparative data between the experimental and historical control groups at the 6–8-week time points: (1) The total number of dogs evaluated. (2) Mean functional score. (3) Standard error of the mean. (4) Range of functional scores (1–5 scale for individual measures of outcome). (5) Comparative statistic; significance set at p ≤ 0.05. The least significant p value of the three comparisons is given for either control versus PEG or control versus P 188. Note that all of the comparisons were significant at p ≤ 0.05.
study. The possible range of an individual dog’s total neurological score was 4 (a completely paraplegic dog) to 20 (a normal dog). Fifteen of the 24 control dogs (62%) remained neurologically complete paraplegics at 6–8 weeks after decompressive surgery and corticosteroid treatment—all assigned a neurological score of 4. Only 16% (3 of 19) PEG-treated dogs remained completely paraplegic at the end of 6–8 weeks and 44% of the P 188–treated dogs. Though a somewhat subjective observation, three or four of the polymer-treated dogs had recovered so completely that any remaining deficit could only be determined by a trained observer and/or a thorough neurological examination. This overall level of performance was never observed in control dogs in previous study.

Electrophysiology and Bladder Management

Eleven of the 12 PEG-treated dogs recruited to the Purdue Center underwent electrophysiological evaluation. SSEP testing was used to assess the recovery of nerve conduction through the lesion (Fig. 2). Sixty-three percent (7 of 11) of these cases were recorded to have positive SSEPs. All four dogs scoring above the median TNS of 12 showed a clear recovery of conduction. In contrast, of the 10 control dogs from the 1993 study, none recovered SSEP conduction by 6 months post-injury (Borgens et

FIG. 2. Electrophysiological tests for conduction through the spinal cord injury—the somatosensory evoked potential. To record ascending evoked potentials, scalp needle electrodes were inserted subcutaneously over the somatosensory cortex contralateral to the side stimulated, while reference electrodes were inserted on the opposite side between the mastoid and the pinna of the ear. The placement of recording electrodes was facilitated by stimulation of the median nerve at the outset, a neural circuit “above,” and unaffected by, the spinal cord injury. (A,B) A copy of a complete set of SSEP recordings are shown. The lower group of waveforms in these pairs are the three individual trains of 200 stimulations as described in methods, and the upper waveform is the averaged evoked SSEP. (C) Only such averaged SSEPs are provided. In A, note the clear evoked potential recorded approximately 10 msec after stimulation of the median nerve of the foreleg. In B, an electrical recording is shown displaying three trains of stimulation, as well as the averaged SSEP as in A. This record was in response to stimulation of the tibial nerve in the same paraplegic dog providing the record in A, approximately 4 days post-injury. The complete elimination of SSEP conduction through the lesion shown in B is characteristic of all neurologically complete paraplegic dogs. SA, stimulus artifact; time base, 5 msec/div; sensitivity, 1.25 μV/div.
al., 1993). This failure to recover measurable SSEPs was confirmed and reported in 1999, where only two of 14 control dogs recovered SSEPs by 6–8 months.

Communication was maintained with owners in the PEG study. Owners reported that 13 of the 19 PEG-treated dogs were continent and did not require bladder expression. None of these dogs were rehospitalized for urinary tract infection, which is the case if the dog remains incontinent, and is not manually expressed. The other dogs were of a group of PEG-treated dogs that exhibited the least recovery at the end of the study, and continued to require bladder management.

**DISCUSSION**

The acute spinal cord injuries evaluated in this study were confirmed to be due to explosive disk rupture and are referred to in dogs as Hansen type 1 lesions—as opposed to the slow progressive protrusion of the disk with time (Hansen type 2; Hansen, 1952). Sudden onset of neurologically complete paralysis, often induced by physical activity in condrodytrophic breeds (Horelein, 1979), accompanied by the aforementioned radiological signs represents catastrophic spinal cord injury in the canine (Hansen, 1952; Horelein, 1979). Dogs, as humans, present to the clinic in various stages of health after severe neurological injury. Radiology confirmed that sometimes disk material may have pierced the dura and the parenchyma of the cord in addition to compressing it (Borgens et al., 1993; Borgens, 2003). This subset of traumatic disk compression injury is comparable to “burst fractures” in humans. As long as conventional procedures for acute care were adhered to, a “slow push” injection of these polymers did not produce any detectable untoward change in the dog’s clinical status. We have also injected PEG into 5 dogs in an attempt to treat severe paraplegia secondary to fracture/dislocation of the spine after automobile impact (data not shown). These are truly impressive spinal injuries in which the overall status of the animal is very critical—a condition not often matched in severity by that typically observed in humans. Injection of PEG was not associated with any unwanted side effects in even these cases. PEG has a long history of safety in both human and animal testing (Shaffer and Critchfield, 1947; Smyth et al., 1947; Shaffer et al., 1948; Principe, 1968; Carpenter et al., 1971; Working et al., 1997), and P 188 has been used in clinical trials for various conditions in human medicine as well (Borgens, 2003).

Given our experience testing several different experimental therapies in dogs (and their control groups) the functional responses to PEG administration in neurologically complete paraplegics dogs was both unexpectedly rapid and relatively complete at best. As suggested in the Results, our impression of P 188 is that it may not be as effective as PEG in treating SCI, particularly in restoring voluntary walking, though the statistical comparisons of TNS between the two polymers does not reveal any difference between them (Table 1). Subtle differences between these polymers can not be easily discriminated in clinical study of dogs given the relatively coarse behavioral and non-invasive methods compared to that used in experimental laboratory animals. A randomized, blinded clinical study in dogs, complete with a contemporaneous control group, may also benefit the decision as to which polymer is more efficacious.

The status of bladder continence due to paraplegia is problematic in dogs just as in man. Owners do not easily confuse changes in continence, since it represents a major behavioral loss in the dog’s training and is the most common reason given for euthanasia of their pets. Moreover, changes in the status of continence in paraplegic animals is usually noted and followed by attending clinicians since a consistent failure of owners to manually express the bladder of incontinent dogs leads to readmission for urinary tract infection. On the basis of owner surveys, we suggest polymer therapy may also improve continence. We offered owner communications in the Results as preliminary evidence that PEG administration likely enabled improved urinary continence.

**Previous Laboratory Animal Experiments**

Using an adult guinea pig SCI model, we have reported an in vivo recovery of (a) the cutaneous trunci muscle reflex (a sensorimotor reflex dependent on the integrity of the ventral white matter) and (b) conduction of evoked potentials through the lesion in response to PEG administration. These results were achieved when PEG application was made directly to the exposed spinal cord lesion after durotomy or by subcutaneous injection (Borgens and Shi, 2000; Borgens et al., 2002; Duerstock and Borgens, 2002). Administration of PEG, as well as P 188 (Borgens et al., 2004) through the vasculature was just as effective as a topical application to the exposed spinal cord. The overall responses were not reduced by a delay in application of approximately 8 hours post injury (Borgens and Bohnert, 2001; Borgens, 2003). A specific targeting of the hemorrhagic spinal cord lesion by PEG occurs following injection of fluorescently labeled polymer. Labeling was completely absent—or barely detectable—in subpial regions of normal undamaged spinal cord near the lesion in the same animals (Borgens and Bohnert, 2001).

**Mechanisms of Action**

In isolated spinal cord, the recovery of electrophysiological function after compression occurred within min-
cludes of a single PEG application, suggesting an initial and rapid interaction with the membranes of damaged cells restoring excitability (Shi and Borgens, 1999; Shi et al., 1999; Luo et al., 2002). Furthermore, we have reported that PEG is able to anatomically “seal” the membranes of such damaged axons in crushed guinea pig spinal cord using dye exclusion tests. Ordinarily, labels like horseradish peroxidase (HRP; MW ~40,000 Da) and ethidium bromide (MW = 400 Da) move into the axoplasm of crushed or severed axons from the extracellular milieu, but not undamaged cells. An application of PEG to the organ culture prior to exposure to the markers blocked the uptake of these labels (Shi and Borgens, 1999; Luo et al., 2002). We have also used the movement of the enzyme Lactate Dehydrogenase (LDH; MW ~160,000 Da) from the cytoplasm to the extracellular fluid as another indicator of membrane compromise. The presence of PEG in the extracellular fluid after damage markedly reduces the loss of the enzyme from the cell (Luo et al., 2002). Thus, the differences in the molecular weights of these various transmembrane markers suggests that PEG is able to seal or “repair” very small to relatively large membrane breaches. The ability of related polymers to seal the membranes of other types of cells has also been documented by Raphy Lee and colleagues where the leakage of intracellular calcein after electric shock to myocytes was vitiated by polymer application (Lee et al., 2002a).

The actual anatomy of putative “holes” or “breaches” in cell membranes, and the interaction of inorganic polymers with this damage is of interest. We are exploring this using atomic force microscopy (AFM) to image living chick dorsal root and sympathetic ganglion neurons. A precise “nanoslice” of the neuron’s membrane in vitro, is performed and then imaged to immediately document the responses. Unexpectedly, such a minor cut (~50 nm wide; 400 nm deep) leads to a steady loss of degraded cytoplasm—pooling around the cell body on the floor of the culture dish for periods of 20 min or longer (McNally and Borgens, 2004). Presently, we are imaging the interaction of polymers with such membrane lesions in living neurons using AFM.

The exact molecular interaction of hydrophilic polymers and non-ionic surfactants with the components of membranes, and the organization of their aqueous phase, to facilitate membrane fusion is still a matter of conjecture (Lentz, 1994; Lee and Lentz, 1997). Apparently dehydration of the membrane (and/or its imperfections) facilitates the hydrophobic core of the lamellae to become continuous. Rehydration of the membrane after polymer exposure permits the polar forces associated with the water phase to help reorganize the structure of transmembrane elements. Thus, the participation of water, together with the special properties of some inorganic polymers, appears to mediate membrane fusion, sealing, and repair (Borgens 2001, 2003; Maskarinec et al., 2002). The conjugation of various chemical groups to PEG can also confer special properties. For example, the hydrophobic head group of a copolymer, P 188, inserted itself directly into a membrane breach sealing it, but was eventually dislodged when the membrane pressure recovered during the repair/reassembly process (Maskarinec et al., 2002).

Clinical Development

The generally excellent record of PEG usage in various contexts in human medicine, together with the results of this preliminary investigation, suggest a move to the human clinic. We have already begun the process to obtain Institutional Review Board approval for a Phase one human clinical trial at Indiana University Medical Center, Division of Neurosurgery, Indianapolis, Indiana simultaneously with initial inquiry to federal regulatory officials at the U.S. Food and Drug Administration.

It is interesting to point out that all study dogs, polymer-treated or the historical controls, received preoperative injections of methylprednisilone sodium succinate—which has been considered a “standard of care” in the veterinary management of SCI as it has been in human cases of SCI. Thus, we cannot separate possible interactions between these intravenous agents in this study, only suggesting we did not detect any unacceptable responses to dual administration. The benefits versus the risks of MPSS is now controversial in both fields of medicine (Borgens, 2003; Coates et al., 1995; Pointillart et al., 2000; Short et al., 2000). We think it possible that intravenous PEG administration may replace the use of MPSS as a “standard of care” in the acute phase of the injury if continued clinical testing supports its safety record (Working et al., 1997) and provides further data indicating a benefit to the patient.

Finally, we emphasize that one must exert the usual caution in considering the comparisons provided in this text given that the control groups were not contemporaneously treated or evaluated. We provide only some of the many possible statistical comparison to historical results, to give the reader an indication of both the trends, and likely merits, of the treatment. Scientifically valid comparisons can only be achieved in our view through the conduct of a randomized, blinded, and controlled study, which is currently being organized.

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