Oxygenated fluorocarbon perfusion as treatment of acute spinal cord compression injury in dogs

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Experiments were conducted to determine the therapeutic value of subarachnoid perfusion of the traumatized dog spinal cord with the fluorocarbon, Fluosol-DA (20%). Control dogs without lesions, but which had durotomy, subarachnoid catheter placement, and saline irrigation for 4 hours, did not have any residual neurological deficit. A series of 41 dogs underwent an acute spinal cord compression using an epidural balloon inflated to a pressure of 160 mm Hg and maintained for 1 hour. Treatment included durotomy only (11 dogs), durotomy with saline perfusion at room temperature (15 dogs), and durotomy with oxygenated Fluosol-DA perfusion at room temperature (15 dogs). The dogs underwent daily grading of neurological status for a 60-day period. Dogs undergoing perfusion of the spinal cord with either saline or oxygenated Fluosol-DA had significantly improved motor recovery (p < 0.004) compared with dogs undergoing durotomy only. Perfusion with oxygenated Fluosol-DA resulted in significantly better motor recovery (p < 0.05) than did perfusion with normal saline. Microscopic examination of the traumatized spinal cords failed to reveal a substantial difference between the three groups. However, dogs with better functional results tended to have less destruction of the white matter. Hemorrhagic necrosis of the central gray matter was consistently observed in all traumatized spinal cords.

KEY WORDS • oxygenated fluorocarbon • spinal cord compression injury • spinal cord perfusion • oxygen
TABLE 1
Summary of experimental groups

<table>
<thead>
<tr>
<th>Group No.</th>
<th>Procedure</th>
<th>No. of Dogs</th>
</tr>
</thead>
<tbody>
<tr>
<td>unlesioned groups</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1A</td>
<td>durotomy only</td>
<td>3</td>
</tr>
<tr>
<td>1B</td>
<td>durotomy &amp; subarachnoid placement of catheters for 4 hrs</td>
<td>5</td>
</tr>
<tr>
<td>1C</td>
<td>durotomy, subarachnoid placement of catheters &amp; saline perfusion for 4 hrs</td>
<td>4</td>
</tr>
<tr>
<td>lesioned groups</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>durotomy only</td>
<td>11</td>
</tr>
<tr>
<td>3</td>
<td>durotomy &amp; saline perfusion (22°C) for 4 hrs</td>
<td>15</td>
</tr>
<tr>
<td>4</td>
<td>durotomy &amp; oxygenated fluorocarbon perfusion (22°C) for 4 hrs</td>
<td>15</td>
</tr>
</tbody>
</table>

Femoral artery cannulation, and at 1 hour and 3 hours after release of spinal cord compression.

Following removal of the spinous processes of T-12, T-13, and L-1, a burr hole was made in the middle of each of the neural arches. The epidural fat was removed at the T-13 burr-hole site. A thin layer of bone was left intact at the bottom of the burr holes at T-12 and L-1 to keep the biomechanics of cord injury as consistent as possible with previous studies done in this laboratory. To facilitate catheter placement, the burr holes at T-12 and L-1 had to be extended laterally. Care was taken to minimize blood loss. A compression injury of the spinal cord was then produced by a balloon compression technique described previously. The intraspinal balloon was inflated to a pressure of 160 mm Hg, and this pressure was maintained against a standard mercury column for 1 hour.

Following release of compression, the layer of bone at the bottom of the burr holes at T-12 and L-1 was removed. The dura was opened in a cruciate fashion at each burr-hole site. At the end of the operative procedure, Gelfoam was placed over the open dura. The wound was then closed in layers.

Due to preparations required for the different treatments, particularly for the fluorocarbon perfusion, the treatment group was known at the time of injury. However, there was no preselection of animals to any group. Care was taken to standardize the cord lesion. Surgery was performed by two individuals, each producing a lesion and administering treatment to approximately one-half of the animals in each group.

**Experimental Groups**

**Unlesioned Operative Control Group.** The control group of 12 dogs did not undergo balloon compression. This group (Group 1) was divided into three arms (Table 1). Group 1A included three dogs that underwent laminectomy and were kept asleep for 4 hours prior to wound closure. During this time, moistened cottonoid strips were placed epidurally to prevent dehydration. This was done in all nonperfused groups.

There were five dogs in Group 1B. In these animals, after laminectomy and durotomy, small polyethylene catheters (PE90) were placed at a distance of 1 to 2 cm along the lateral aspect of the subarachnoid space via the T-12 and L-1 burr holes. The direction of placement was toward the T-13 burr hole. One catheter was placed on the left side and the other catheter on the right side of the spinal cord. The catheters were tied to the paravertebral muscles to ensure position maintenance (Fig. 1). The animals were kept asleep for 4 hours prior to wound closure.

Group 1C contained four dogs. In these animals, after laminectomy, durotomy, and catheter placement, the cord underwent subarachnoid perfusion with normal saline at room temperature for 4 hours as described in Group 1C, but beginning 30 to 45 minutes after release of compression.

*H.R. flow inducer, Type MHRE200, manufactured by Watson-Marlow, Cornwall, England.*
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FIG. 2. Graph showing a summary of the results in the three nonlesioned control groups.

Group 4 included 15 dogs. After laminectomy, production of the lesion, and durotomy, subarachnoid perfusion with oxygenated Fluosol-DA (20%)† was carried out for 4 hours. Pure oxygen was bubbled through the fluorocarbon solution for several minutes before perfusion and during the perfusion procedure. Perfusion was started 30 to 45 minutes following release of balloon compression, and was performed by gravity flow as described for Group 1C, but the flow rate was only 16 to 25 ml/min due to the high viscosity of the fluorocarbon solution.

Postoperative Care
All dogs were given antibiotics (penicillin and dihydrostreptomycin) for 5 days in the postoperative period. A sufficient bedding of shavings was placed on the floor to try and prevent the dogs from forming decubitus lesions. Any decubiti that did develop were treated with nitrofurazone cream. Bladders were expressed using abdominal compression twice a day.

The animals were assessed daily by at least two experienced examiners, neither of whom knew the other's findings or which treatment the animals had received. Assessments continued for 60 days, using a modification of our previous evaluation table (Table 2).

Postmortem Examination and Histology
At 60 days, the animals were anesthetized and killed by exsanguination. A laminectomy was performed from two levels cephalad to two levels caudad to the site of injury. The cord was inspected, removed, and fixed in Millonig's solution. The tissue was embedded in paraffin, sliced into coronal or longitudinal sections (8 μ); and stained with hematoxylin and eosin, neutral red, and/or Luxol fast blue stains. The microscopic sections were assessed by an individual completely blind to the treatment-group allocation.

Statistical Analysis
An analysis of the results of functional recovery assessments was made using the Mann-Whitney U-test of orthogonal comparisons. Fluosol-DA perfusion (Group 4) was compared to saline perfusion (Group 3), and the results of perfusion using Fluosol-DA or saline (Groups 3 and 4) were compared to the results of durotomy only (Group 2).

Animals that did not survive for 60 days, or injured animals that were graded at more than a score of 1.0 on the 1st postoperative day, were excluded from the experiment and hence from any statistical analysis.

Results
All nonlesioned dogs were able to walk by the 3rd postoperative day and to run well at 60 days (Fig. 2

<table>
<thead>
<tr>
<th>Score</th>
<th>Observation</th>
</tr>
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<tbody>
<tr>
<td>1.0</td>
<td>no voluntary movements</td>
</tr>
<tr>
<td>2.0</td>
<td>voluntary movements present</td>
</tr>
<tr>
<td>2.5</td>
<td>tries to stand; hindlegs successfully elevate buttocks off ground</td>
</tr>
<tr>
<td>3.0</td>
<td>stands &amp; takes several steps with hindlegs</td>
</tr>
<tr>
<td>3.5</td>
<td>walks well</td>
</tr>
<tr>
<td>4.0</td>
<td>runs</td>
</tr>
<tr>
<td>4.5</td>
<td>runs well</td>
</tr>
<tr>
<td>5.0</td>
<td>normal</td>
</tr>
</tbody>
</table>

* Modified Tarlov scale.
and Table 3). Durotomy, catheter placement, and perfusion were moderately easy to do in these animals.

At no time was there a discrepancy of more than one rating score between the two assessors. Two (18%) of the 11 animals treated by durotomy only (Group 2) were able to walk at the end of the assessment period. Seventeen (57%) of the 30 animals treated with fluorocarbon or saline perfusion were able to walk at the end of the assessment period. The combined result following perfusion with either saline or Fluosol-DA demonstrated highly significant levels of recovery (p < 0.004) when compared with durotomy only. Severity of the lesion was attested to by the mean motor performance of the durotomy-only group (Group 2), which stabilized after 6 weeks at a level indicating inability to elevate the buttocks from the ground.

Six (40%) of the 15 animals treated with saline perfusion (Group 3) were able to walk, whereas 11 (73%) of the 15 animals treated with oxygenated fluorocarbon (Group 4) were able to walk at the end of the assessment period. The difference between these two groups also was significant (p < 0.05).

The group treated with Fluosol-DA (Group 4) recovered faster than the saline-perfused or durotomy-only groups (Fig. 3). Also, the fluorocarbon-treated group was the only one that displayed a final mean motor recovery greater than a score of 3, which indicates the ability to walk (Table 3).

During the course of the experiment, two of the dogs undergoing durotomy only following spinal cord injury were excluded because some motor function was present on the 1st postoperative day. It was discovered that the pressure gauge monitoring the intraspinal balloon was not working properly at the time of those two experiments. No dog in Groups 3 and 4 (perfused cords) showed this rapid return of motor recovery. In addition, two of the untreated (durotomy only) dogs had to be killed in the 3rd postoperative week due to the severity of the decubitus ulcers which they developed. Only one animal died 1 week after perfusion with saline, and no animal treated with fluorocarbon died in the postoperative period.

At one time, perfusion was carried out using a pump which infused the subarachnoid space at rates which we varied between 180 and 300 ml/min. Such high flow rates were observed to be deleterious to the anatomical and functional integrity of the spinal cord (Fig. 4). In contrast, no deleterious effects were observed resulting from the gravity technique of perfusion used in this study.

### TABLE 3

<table>
<thead>
<tr>
<th>Group No.</th>
<th>No. of Dogs</th>
<th>Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>1A</td>
<td>3</td>
<td>4.83</td>
</tr>
<tr>
<td>1B</td>
<td>5</td>
<td>5.0</td>
</tr>
<tr>
<td>1C</td>
<td>4</td>
<td>5.0</td>
</tr>
<tr>
<td>2</td>
<td>11</td>
<td>2.27 ± 0.85 (2 &gt; 3.0)</td>
</tr>
<tr>
<td>3</td>
<td>15</td>
<td>2.7 ± 0.56 (6 &gt; 3.0)</td>
</tr>
<tr>
<td>4</td>
<td>15</td>
<td>3.03 ± 0.61 (11 &gt; 3.0)</td>
</tr>
</tbody>
</table>

*For a summary of experimental groups see Table 1.
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Histological examination of cross sections of the spinal cords consistently demonstrated evidence of previous hemorrhagic necrosis of the central gray matter. The sections illustrated are taken from, and fairly representative of, each group of dogs. Dogs with a better functional recovery tended to have less white matter destruction, although the average histology of the various groups showed no significant difference between the groups (Fig. 5).

Discussion

The fluorocarbon solution (Fluosol-DA 20%) used in this experiment consists of an emulsion of particles, 90% by weight of which are less than 0.2 μ in diameter and none of which are greater than 0.6 μ. The combined surface area of the particles is $1.82 \times 10^6$ sq cm/liter available for oxygen transport. This is about 100 times more than blood, which contains a surface area of $1.86 \times 10^6$ sq cm oxygen-carrying particles per liter. The time taken for oxygen exchange or release by the fluorocarbon particles is twice as fast as hemoglobin, and the time of CO₂ transfer is a few milliseconds.²⁶ Fluosol-DA emulsion has been used successfully for exchange transfusions in various mammals, including rabbits, rats, dogs, monkeys, and man.²⁰,²⁶ It has been generally accepted that perfluorocarbons are biologically inert and not catabolized in vivo.⁶ Perfluorocarbon emulsions have also been used for perfusion of isolated brains.⁶,³⁸ These properties of fluorocarbon led us to choose them as a carrier of oxygen to the site of injury.

The fluorocarbon emulsion is stored in the frozen state and requires thawing before use. Consequently, there could be no random allocation of injured animals to the various treatment groups following their injury. However, there was no preselection of animals to any of the treatment groups, and the method of injury was standardized. Despite these precautions, there was still a slight variability in recovery within each lesioned group. Results of the operative controls showed that laminectomy, catheter placement, and saline perfusion via the gravity technique could be performed without leaving the dogs with any neurological deficit.

The pooled results of animals treated with any perfusion at room temperature showed to a highly significant degree that perfusion is better than no perfusion at all (p < 0.004). This supports the conclusion of other studies published previously.⁶,³⁷ At no time did any noticeable side-effect develop as a result of perfusion with saline or oxygenated fluorocarbon using the technique described above.

No significant difference in pathology of cord tissues was detected. The gray matter was severely damaged in all groups with spinal cord compression. The white matter also exhibited extensive destruction in most injured animals, although there was a tendency for treated animals to show less damage to the white matter than the untreated controls. This observation is supported by the significant differences in the functional recoveries of the injured groups.

It is not known whether the enhanced recovery of the perfusion groups over the control group is due to hypothermia as reported by others¹,¹²,²¹ (both perfusates were at room temperature), or to washout of noxious substances, or even to a combination of these factors. Recent publications questioning the existence of noxious substances,²,³⁶ and the fact that our group with the slower rate of perfusion did significantly better (p < 0.05) may be evidence that the beneficial effect observed in this experiment was not due to washout of noxious substances.
Although treatment using oxygenated fluorocarbon was originally attempted in order to improve the availability of oxygen at the site of injury, it cannot be assumed that the improvement of the dogs treated in this fashion compared with dogs treated using saline perfusion and/or durotomy only was due to any difference of oxygenation at the site of injury. The fluorocarbons have an osmolality of approximately 410 mOsm/liter in contrast with saline or cerebrospinal fluid (CSF), which have an osmolality in the range of 300 mOsm/liter. Such a difference in osmolality may have caused a significant difference in the recovery of the two perfused groups. However, Richardson and Nakamura indicated that the hyperosmolar agent, mannitol, given intravenously, was of questionable histological benefit in the treatment of cats with spinal cord injury.

Doss, et al., reported that intrathecal injection of oxygenated fluorocarbon increased CSF oxygen tension by a factor of 5, but also produced a gradual CSF acidosis. These authors found that, by replacing small amounts of CSF in cats with oxygenated fluorocarbon, they were able to observe a significantly reduced drop in cerebral oxygen tissue pressure induced by anoxic anoxia.

In 1972, Kelly, et al., reported improved locomotor recovery in cord-injured dogs in which they improved tissue oxygen pressure at the site of the spinal cord injury using hyperbaric oxygen therapy. In rats with cord transection, Gelderd also showed improvement in motor recovery following hyperbaric oxygen therapy. It would be tempting to postulate that the improvement in motor recovery observed in the fluorocarbon-treated group was due in part to an increased oxygenation of the injured cord. However, our experiment gives no direct evidence to prove this.
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In view of the encouraging results obtained, further investigation will have to be conducted. In contrast to the results of Gelderd and Kelly, other authors have found that hyperbaric oxygen therapy is deleterious and indeed, may cause damage to the central nervous system and other parts of the body. Results from this study indicate that a method of localized treatment aimed at improving oxygenation of the injured spinal cord may be safer than hyperbaric oxygen.

Other factors that may have contributed to the difference in the level of recovery of the two perfused groups are: 1) the difference in perfusion rate (the group of animals that did best had the lower rate of perfusion); and 2) differences in physical or chemical properties, including the fact that the fluorocarbon emulsion may have had a higher affinity for noxious substances at the site of injury.

In conclusion, fluorocarbon perfusion at room temperature is more beneficial in improving the motor recovery of acutely paralyzed dogs than saline perfusion alone, and it appears that fluorocarbons help via a mechanism in addition to room temperature perfusion, possibly improvement in the $pO_2$ at the site of injury. Since a safe technique of perfusion for animal studies has been described, it may be feasible to use this in humans at some time in the future after further studies.

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