Failure of tetracaine to reverse spinal cord injury in the cat

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Beginning 30 minutes after acute spinal cord injury, cats were treated by the administration of continuous spinal anesthesia for 8 hours. This was achieved by the intermittent injection of hyperbaric tetracaine into the subarachnoid space at the site of injury via an indwelling catheter. There were no significant differences in functional recovery or histologically assessed tissue preservation between treated cats and concurrently managed control animals. The indwelling subarachnoid catheter used for drug administration was found to have no significant effect on the spinal cord injury.

KEY WORDS - spinal cord injury • local anesthetic agent • spinal anesthesia

Spinal cord injuries sufficient to cause permanent paraplegia in experimental animals are associated with decreased blood flow in both white and gray matter at the site of injury. Blood flow in the gray matter is essentially obliterated within 1 hour of such injuries, and is followed by complete necrosis. In the white matter, on the other hand, blood flow remains in the normal range for approximately 1 hour and then decreases for at least 8 to 12 hours. At 24 hours following injury, the blood flow to the white matter has been observed by some to return to preinjury levels and by others to remain markedly diminished. Transitory vasospasm has been demonstrated in the injured feline spinal cord and has been postulated as a major cause of reduced white matter blood flow.

Topical local anesthetic agents have been reported to reverse cerebral vasospasm in man and animals. The local anesthetic, lidocaine, reduces cerebral basal metabolism and prevents the efflux of cellular potassium from the globally ischemic brain. It has been suggested that the membrane-stabilizing action of local anesthetic agents may protect ischemic neural tissue.

The present study was designed to evaluate the therapeutic effect of the topical application of a local anesthetic agent to the injured spinal cord.

Materials and Methods
Animal Preparation
Thirty adult male cats, each weighing 3.1 to 6.3 kg, were used in these experiments. The animals were premedicated intramuscularly with xylazine (0.5 mg/kg) and atropine (0.02 mg/kg), anesthetized with ketamine (20 mg/kg intramuscularly), intubated, and ventilated with a Bird respirator and Bain circuit. Anesthesia was maintained with 70% N2O and 30% O2. Halothane, 0.25% to 0.5%, was added during the initial operative phase of each experiment. Intravenous injections of fentanyl (2 μg/kg) were given subsequently as required to ensure adequate anesthetic depth. Following induction of anesthesia, arterial and venous cannulas were placed in the femoral vessels and a No. 8 French Foley catheter was placed in the bladder through a small midline abdominal incision.

Monitoring Methods

The arterial pressure waveform, mean arterial pressure, electrocardiogram, and heart rate were monitored with Hewlett-Packard monitors and displayed on an oscilloscope screen. The end-tidal CO2 was monitored with a Beckman CO2 analyzer and, together with blood gas analyses, was used to maintain the arterial pCO2 between 35 and 40 mm Hg for the duration of the experiment.
Injury Technique

Spinal cord injuries were produced using a modified Allen technique. Briefly, under general anesthesia, a partial laminectomy was performed at L-1, and the spine immobilized by clamping the spinous processes of T-13 and L-2 to the framework of the injury apparatus. A curved stainless steel plate, or anvil, was placed beneath the spinal cord and dura to center the cord and provide a smooth impact surface. The length of time that the anvil remained beneath the cord was recorded. The anvil was then firmly attached to a vented straight vertical brass tube. A 1.4-gm Teflon hammer, contoured to fit the anvil, protruded from the lower end of the brass tube and rested gently on the cord's dorsal surface. The spinal cord was injured by dropping a 10-gm iron weight through the brass tube from a known height. To ensure that only a single impact was delivered to the cord, the weight was caught and held by an electromagnet which was electronically activated on impact. Pancuronium (0.05 mg/kg) was given intravenously just prior to producing the spinal cord injury to prevent movement.

With this technique, complete paraplegia of at least 6 weeks' duration results when magnitudes of 10 gm x 20 cm, or greater, are used to injure the spinal cord. When injury magnitudes are 10 gm x 10 cm, or less, animals regain the ability to walk within 6 weeks.

Experimental Groups

Animals were randomly assigned to one of three experimental groups (Table 1). Animals in Group A received a 10 gm x 20 cm spinal cord injury and were subsequently treated by tetracaine, a long-acting local anesthetic agent, administered to the injury site via a subarachnoid catheter. Animals in Group B received the same injury, while those in Group C received a lesser injury (10 gm x 10 cm). Animals in Groups B and C were treated exactly like those in Group A, except that nothing was injected into the catheters. A comparison of animals in Groups B and C with animals previously injured by identical techniques in this laboratory demonstrates the contribution of the drug administration technique, if any, to the spinal cord injury.

Following spinal cord injury, the dura was retracted from the cord at the injury site with an 8-0 ophthalmic suture, and a small stab wound was made with a No. 11 scalpel blade. A thin Silastic tube (0.025 in. in outer diameter) was then inserted into the subarachnoid space and advanced 1.5 cm in a rostral direction. A piece of Gelfoam was placed over the injury site, and the fascia and skin were temporarily closed with interrupted 2-0 Vicyrl and 4-0 Dexon sutures, respectively.

Subarachnoid injections of 0.5% tetracaine dissolved in 5% dextrose and water were given to Group A animals at 1, 3, and 5 hours after injury. An initial dose of 5 mg was followed by two subsequent doses of 2.5 mg each. This regimen was found in preliminary experiments to provide at least 8 hours of spinal anesthesia in the normal awake cat. All animals in all groups were placed in a 15° head-up position during the time that the subarachnoid catheter was in place. This was done to prevent respiratory paralysis in those animals (Group A) receiving the hyperbaric spinal anesthetic solution. Six hours after injury, each laminectomy incision was opened to confirm the subarachnoid position of the Silastic catheter. The catheter was then removed, a fresh piece of Gelfoam was placed at the injury site, and the wound was resutured. No attempt was made to close the small dural puncture wound. Each animal remained under general anesthesia until final wound closure was completed.

To minimize experimental bias, neither the experimental group to which each animal was assigned, nor the magnitude of the injury, was revealed to the investigator until after the spinal cord had been injured.

Postinjury Management

Postoperatively, animals were kept in a heated cage until fully recovered from anesthesia. They were then housed in groups of six in large animal pens. To prevent

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**TABLE 1**

<table>
<thead>
<tr>
<th>Group</th>
<th>No. of Cats</th>
<th>Injury Magnitude</th>
<th>Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>10</td>
<td>10 gm x 20 cm</td>
<td>tetracaine + catheter</td>
</tr>
<tr>
<td>B</td>
<td>10</td>
<td>10 gm x 20 cm</td>
<td>catheter only</td>
</tr>
<tr>
<td>C</td>
<td>10</td>
<td>10 gm x 10 cm</td>
<td>catheter only</td>
</tr>
</tbody>
</table>

**TABLE 2**

Criteria for scoring motor recovery in cats

<table>
<thead>
<tr>
<th>Score</th>
<th>Motor Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>unable to walk</td>
</tr>
<tr>
<td>1</td>
<td>walks a few steps only</td>
</tr>
<tr>
<td>2</td>
<td>unlimited weak gait</td>
</tr>
<tr>
<td>3</td>
<td>walks with slight weakness</td>
</tr>
<tr>
<td>4</td>
<td>jumps up 3 feet</td>
</tr>
<tr>
<td>5</td>
<td>normal</td>
</tr>
</tbody>
</table>

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§ Yellow Springs 401 probe and Model 73A temperature controller manufactured by Yellow Springs Instrument Co., Yellow Springs, Ohio.
Tetracaine and spinal cord injury

TABLE 3
Experimental variables at time of injury (mean ± standard deviation)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>A</th>
<th>B</th>
<th>C</th>
</tr>
</thead>
<tbody>
<tr>
<td>weight (kg)</td>
<td>4.5 ± 1.1</td>
<td>4.0 ± 0.8</td>
<td>4.2 ± 0.6</td>
</tr>
<tr>
<td>arterial pO2 (mm Hg)</td>
<td>120 ± 20</td>
<td>129 ± 13</td>
<td>133 ± 17</td>
</tr>
<tr>
<td>arterial pCO2 (mm Hg)*</td>
<td>39 ± 2</td>
<td>37 ± 2</td>
<td>36 ± 2</td>
</tr>
<tr>
<td>mean arterial pressure (MAP) (mm Hg)</td>
<td>86 ± 13</td>
<td>94 ± 28</td>
<td>88 ± 13</td>
</tr>
<tr>
<td>peak increase in MAP (% of preinjury MAP)</td>
<td>17 ± 20</td>
<td>15 ± 14</td>
<td>17 ± 13</td>
</tr>
<tr>
<td>esophageal temperature (°C)</td>
<td>38.2 ± 0.9</td>
<td>37.6 ± 1.2</td>
<td>37.8 ± 0.9</td>
</tr>
<tr>
<td>time anvil under cord (min)</td>
<td>4.5 ± 1.0</td>
<td>3.5 ± 0.8</td>
<td>4.5 ± 2.6</td>
</tr>
</tbody>
</table>

* Analysis of variance (Student-Newman-Keuls test) reveals a significant difference between Groups A and C (p < 0.05).

pressure sores, the floors of the pens were covered with fine wood shavings. Two animals, one from Group B and one from Group C, developed small decubitus ulcers.

Suprapubic catheters were drained daily. When bladder output was less than 50 ml on 3 consecutive days, the catheters were removed. Daily manual expression was then carried out. Suprapubic catheters remained in the bladders of Group A, B, and C animals for 18 ± 8, 15 ± 4, and 14 ± 5 days (mean ± standard deviation), respectively. Analysis of variance revealed no significant differences between these mean durations.

The first 19 animals (seven, eight, and four cats from Groups A, B, and C, respectively) were given daily intramuscular injections of penicillin (100,000 units) and streptomycin (0.125 mg) for 5 days following injury. Subsequently, they received daily oral administration of sulfisoxazole (Gantrisin, 125 mg) until the suprapubic catheters were removed. The final 11 animals (three, two, and six cats from Groups A, B, and C, respectively) were given an intramuscular injection of penicillin (100,000 units) and streptomycin (0.125 mg) at the time of surgery only. Catheter or midstream urine samples were cultured three times weekly. Fifty percent of the animals developed persistent urinary infections following injury, but no significant difference was found between the infection rates of animals treated with daily antibiotics and those treated with a single intraoperative dose (chi-square, p > 0.30).

Assessment of Recovery

Each animal was assessed daily for 6 weeks by an individual unaware of the treatment group to which the animal had been assigned. During that time, the 1st day that an animal was observed to elevate its hindlimbs and take a few steps was recorded. On the 42nd day after injury, hindlimb motor function of all animals was recorded according to the criteria of Table 2.

Histological Study

Six weeks after injury, all animals were placed under general anesthesia and perfused through the left ventricle (with the right auricle incised) with 1 liter of normal saline followed by 2 liters of 3.3% buffered formalin. The spinal cord from T-11 to L-4 was then removed, pinned to its full length on wax, and placed in a bath of 10% buffered formalin. Spinal cord cross-sections from the site of maximum injury, and from sites two segments rostral and caudal to this, were prepared for study by light microscopy and stained with hematoxylin and cosin, and Luxol-fast blue dye.

The area occupied by the white matter on each section was determined with the aid of a computer. Briefly, the reflection of a graphics tablet and stylus was superimposed on a low-power microscope image of the spinal cord section. The white matter contour was traced with the stylus image, and the area encompassed by the stylus on the graphics tablet was determined by a computer program.* The amount of normal-staining white matter remaining at the site of maximum injury was expressed as a percentage of the average white matter area measured on sections taken rostral and caudal to the level of injury.

Spinal cord sections were prepared and white matter measurements were made without prior knowledge of the group of animals from which each cord was obtained.

Statistical Significance and Sample Size

In this laboratory, a spinal cord injury of 10 gm x 20 cm magnitude produces permanent paraplegia in 95% of animals. Given this false recovery rate of 5%, a sample size of 10 is needed to be 80% certain (β = 0.2) of detecting (at the 5% level of significance, α = 0.05) a treatment that would enable 60% of animals to walk within 6 weeks of injury.40

Results

Mean values of basic physiological parameters immediately prior to injury and of peak changes in blood pressure following injury are given in Table 3. The mean duration of time that the anvil was positioned

* Polar Bear Morphometric Program distributed by Computerland, Vancouver, British Columbia, Canada.
beneath the cord is given in the same table. There were no significant differences between groups for any parameter, except that the mean value of the arterial pH of Group A differed significantly from that of Group C (analysis of variance, p < 0.05).

Functional recovery, assessed 6 weeks after injury, is shown in Table 4. The final recovery scores of animals in Group C were significantly different from those of either Group A or B. Differences in recovery scores between animals of Groups A and B were not significant. One animal in Group A died unexpectedly on the 28th day postinjury. The suprapubic catheter was still in situ at the time of death and a large area of hemorrhagic infarction was noted in the bladder wall at necropsy. This animal had been treated daily with antibiotic therapy. Marked muscle wasting was evident in the hindlimbs and it had demonstrated no tendency to walk. It was assigned a final score of zero.

Histological assessment of spinal cord tissue preservation at the site of maximum injury is given in Table 5. The gray matter was completely destroyed in all animals. Group C animals had a significantly greater amount of normal-staining white matter at the site of maximum injury than either Group A or B animals. There was no significant difference between the white matter remaining in Group A or B animals and that remaining in cords previously injured by 10 gm x 20 cm injuries in this laboratory. Similarly, white matter preservation in Group C animals was not statistically different from that in animals subjected to 10 gm x 10 cm injuries in a previous study.

Tables 4 and 5 show that the topical administration of tetracaine to the injured spinal cord affords no significant therapeutic benefit. Only one treated animal was able to stagger a few steps by the 35th day.

All Group B animals were completely paralyzed 6 weeks after injury and conformed to the model’s expectation for this injury magnitude. It can be concluded, therefore, that the presence of the subarachnoid catheter did not improve recovery following spinal cord injury. One Group C animal failed to walk within the 6-week assessment period following injury. This suggests that the subarachnoid catheter may have added to the spinal cord injury. However, this occurrence was not statistically significant.

### TABLE 4

**Recovery scores 6 weeks after injury***

<table>
<thead>
<tr>
<th>Group</th>
<th>Final Recovery Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>9</td>
</tr>
<tr>
<td>B</td>
<td>10</td>
</tr>
<tr>
<td>C</td>
<td>1</td>
</tr>
</tbody>
</table>

*Group C is significantly different from Group A and Group B (Wilcoxon-Mann-Whitney test, p < 0.01). For definition of motor recovery see Table 2.

### TABLE 5

**Normal-staining white matter remaining at site of maximum injury (percent)**

<table>
<thead>
<tr>
<th>Group</th>
<th>Mean</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>1</td>
<td>0–3</td>
</tr>
<tr>
<td>B</td>
<td>2</td>
<td>0–14</td>
</tr>
<tr>
<td>C</td>
<td>15</td>
<td>0–32</td>
</tr>
</tbody>
</table>

*Group C is significantly different from Groups A and B (Wilcoxon-Mann-Whitney test, p < 0.01).

### Discussion

Topically applied local anesthetic agents have been reported to reverse vasospasm in cerebral vessels and penetrate the substance of the spinal cord following injection into the subarachnoid space. The route of penetration is presumably the perivascular Virchow-Robin spaces which accompany blood vessels as they enter or leave the spinal cord. Perivascular spaces have been observed in the spinal cord around typical mammalian arterioles and venules and 40% of feline capillaries. Drugs injected into the subarachnoid space would, therefore, have direct access to the surface of intramedullary blood vessels.

All local anesthetics in concentrations of $1 \times 10^{-5}$ M or greater, relax drug-induced arterial contractions. The concentration of tetracaine injected into the subarachnoid space in the present study was $1.7 \times 10^{-5}$ M, well in excess of that needed to relax constricted vessels.

In man, tetracaine produces complete spinal anesthesia for approximately 2½ hours. Spinal anesthesia in the cat was found in preliminary experiments to be of similar duration. In the dog, complete motor blockade lasted more than 2 hours following the subarachnoid injection of 1 ml of 0.5% hyperbaric tetracaine. The subarachnoid injection schedule used in the present study, therefore, should have been adequate to produce a continuous vasodilating action within the spinal cord for approximately 7 to 8 hours following injury.

The above considerations suggest that sufficient tetracaine was administered in the postinjury period to reverse vasospasm in the injured spinal cord. The failure of this treatment to improve neurological outcome suggests that vasospasm is not the cause of white matter destruction. This conclusion is based on two major assumptions, neither of which, however, has been substantiated. The first is that perivascular spaces remain open following injury, allowing access of the tetracaine to the vasospastic vessels within the cord. The second is that reversal of vasospasm for approximately 8 hours is sufficient to prevent its recurrence.

In addition to assessing the effects of tetracaine on spinal cord injury, this study establishes the present technique of administering subarachnoid drugs as an innocuous procedure with respect to the spinal cord injury model. It has been reported that durotomy alone improves recovery following spinal cord injury in the


Tetracaine and spinal cord injury

**TABLE 6**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Experimental Group</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>I</td>
</tr>
<tr>
<td>weight (kg)</td>
<td>4.3 ± 0.6</td>
</tr>
<tr>
<td>arterial pO2 (mm Hg)</td>
<td>147 ± 30</td>
</tr>
<tr>
<td>arterial pCO₂ (mm Hg)</td>
<td>30 ± 2</td>
</tr>
<tr>
<td>mean arterial pressure (MAP) (mm Hg)</td>
<td>96 ± 23</td>
</tr>
<tr>
<td>peak increase in MAP (% of preinjury MAP)</td>
<td>19 ± 8</td>
</tr>
<tr>
<td>esophageal temperature (°C)</td>
<td>37.5 ± 1.2</td>
</tr>
<tr>
<td>time anvil under cord (min)</td>
<td>3.6 ± 0.9</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>II</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>4.0 ± 0.3</td>
</tr>
<tr>
<td></td>
<td>144 ± 16</td>
</tr>
<tr>
<td></td>
<td>31 ± 2</td>
</tr>
<tr>
<td></td>
<td>93 ± 16</td>
</tr>
<tr>
<td></td>
<td>20 ± 26</td>
</tr>
<tr>
<td></td>
<td>36.9 ± 0.4</td>
</tr>
<tr>
<td></td>
<td>4.0 ± 0.7</td>
</tr>
</tbody>
</table>

*Values represent the mean ± standard deviation. For description of experimental groups see Discussion.*

dog, whereas, in the monkey, it was found to have no effect.

The Group B protocol was included in the present study to rule out the potentially beneficial effect of incising the dura to allow insertion of the subarachnoid catheter. That no improvement resulted from this procedure is evident from the lack of recovery of animals in Group B (Table 4).

Introduction of a catheter into the subarachnoid space could have aggravated the spinal cord injury. That this did not occur, however, is evident from examination of the final recovery scores of animals in Group C. With one exception (not statistically significant), all regained the ability to walk as expected for this magnitude of injury.

A significant difference in the arterial pCO₂ between Groups A and C is noted in Table 3. As CO₂ affects blood flow in the central nervous system, it is possible that differences in final recovery scores between Groups A and C could be explained on the basis of variations in the arterial pCO₂. In addition, we have recently learned that the normal arterial pCO₂ in the awake cat at 37°C is 30 mm Hg rather than 40 mm Hg, as in most other animals including man. We have subsequently confirmed this by measuring the pCO₂ of femoral arterial samples drawn from awake, resting, paraplegic cats.

To assess the possible effect of mild hypercapnia on recovery in this spinal cord injury model, 10 animals were randomly assigned to one of two injury groups. The five animals in Group I were given 10 gm x 10 cm injuries and those in Group II received 10 gm x 20 cm injuries. All injuries were produced exactly according to the protocol previously described, except that normocapnia was maintained throughout the experiments. Tables 6, 7, and 8 show the experimental variables at the time of injury, the final recovery scores, and white matter preservation in these two groups.

There were no significant differences between groups for any variable in Table 6 (analysis of variance). When compared with animals previously injured in this laboratory under conditions of mild hypercapnia, no significant differences in the ability to walk 6 weeks after injury or in white matter preservation were found at either injury magnitude (Mann-Whitney U-test). The injury-recovery curve previously determined did not shift with the change from mild hypercapnia to normocapnia. These results suggest that the difference in arterial pCO₂ noted in Table 3 did not appreciably affect the recovery of animals in Groups A or C.

We conclude that the intrathecal administration of tetracaine at the site of spinal cord injury via subarachnoid catheter does not improve functional or histological recovery. Furthermore, the insertion of a subarachnoid catheter following injury does not alter the expected outcome in this spinal cord injury model.

**Acknowledgments**

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