Naloxone Therapy During Focal Cerebral Ischemia Evaluation in a Primate Model

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SUMMARY Conflicting reports have appeared in the literature regarding the effect of the opiate antagonist naloxone on ischemic neurologic deficits. We report the results of a study using naloxone in our model of focal cerebral ischemia in the awake primate. A total of 14 adult baboons were subjected to six-hour occlusion of the left middle cerebral artery (MCA). Seven animals served as controls and seven received treatment with naloxone (5 mg/kg) beginning 30 min after MCA occlusion and continuing until two hours after reperfusion. All animals developed profound hemiparesis and homonymous hemianopsia within seconds of inflating the MCA occluder.

Acutely, therapy with naloxone partially reversed ischemic neurologic deficits in five of the seven treatment animals. Within minutes of receiving the loading dose of naloxone, responding animals were more alert and demonstrated improvements in motor function.

Naloxone did not affect mortality: Three animals in the treatment group and two in the naloxone group died secondary to malignant intracranial pressure within 48 hours of the ischemic episode. In animals surviving the ischemic insult however, treatment with naloxone significantly improved neurologic outcome at 10 days (p < 0.05). Neuropathologic examinations in these animals revealed amelioration of ischemic tissue damage, with three of the five suffering only small focal areas of infarction. (All control animals suffered large infarcts of the MCA territory.) Our results verify that naloxone can reverse ischemic deficits, and more importantly may improve the outcome from focal ischemic insults.

In order to investigate these issues, we studied the effects of naloxone after MCA occlusion in our standardized primate model. Specifically, we sought to determine whether naloxone would reverse acute neurologic deficits during and/or improve the final outcome after six hours of MCA occlusion.

Material and Methods

A total of 14 adult baboons (Papio anibus) were used for this study. Animals weighed from 15 to 28 kg. Both control and treatment groups were subjected to a six-hour period of MCA occlusion. Seven animals were randomly assigned to receive treatment with naloxone and seven served as controls. We have previously reported the details of this experimental model. Briefly, animals were prepared as follows. After induction of general anesthesia with thiopental, the animal was intubated and placed on a volume cycled respirator. Anesthesia was maintained with thiopental infuision. The left orbit and scalp were prepped and draped using sterile technique, and with the aid of an operating microscope, the left MCA was exposed.
through a standard transorbital approach. An inflatable Silastic balloon occluder was then placed around the MCA, with the cuff effecting occlusion positioned immediately adjacent to the origin of the MCA and proximal to the lenticulostriate perforators. The occluder was firmly secured by filling the orbit with methyl methacrylate and the remaining catheter, valve and reservoir, were placed subcutaneously over the skull.

Following operative placement of the occluder, animals were returned to their cages and allowed at least 48 hours to recover before subsequent experimentation. During this recovery period animals were observed daily. Any animal showing evidence of neurologic deficit was excluded from further investigation.

The day prior to planned occlusion, animals were prepared for intensive care monitoring. Under Ketamine anesthesia, percutaneous femoral catheterization was employed to introduce a central arterial pressure line, a Swan-Ganz catheter and femoral venous line. A fiber optic epidural sensor was positioned through a right parietal trephine for continuous intracranial pressure monitoring (Ladd Intracranial Pressure Monitor M1000, Ladd Research Industries, Inc.). EKG leads were also secured for pulse and rhythm analysis.

Animals were placed in a modified primate restraint chair and observed for approximately 24 hours, assuring that they were awake, stable, and remote from the effects of anesthesia at the time of occlusion. Both groups of animals received intravenous maintenance fluids during the period of observation. Controls received no additional therapy. Therapy for the seven animals in the treatment group consisted of a loading dose of naloxone (5 mg/kg) given as an intravenous bolus 30 min after MCA occlusion, followed by constant intravenous infusion of naloxone at 5 mg/kg/hr during the remaining period of occlusion and for 2 hours after reperfusion. Naloxone-HCl was provided courtesy Endo Laboratories in pure crystalline form and prepared in a sterile solution for intravenous administration. Naloxone was dissolved in a solution of 0.9% sodium chloride for a final concentration of 10 mg/ml and filtered free of contaminants.

Neurological exams were performed by laboratory personnel prior to occlusion, post-occlusion and periodically during the period of laboratory observation. Following the acute experiments, animals from both groups were returned to the Department of Animal Facilities and observed in their cages. Final neurological deficits were also secured for pulse and rhythm analysis.

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Neurological exams were performed by laboratory personnel prior to occlusion, post-occlusion and periodically during the period of laboratory observation. Following the acute experiments, animals from both groups were returned to the Department of Animal Facilities and observed in their cages. Final neurological exams were performed on all surviving animals 10 days after the occlusive episode by an expert unaware of the animals' history.

Neurologic scores are based on a normal of 100 points, and are calculated using standardized criteria (fig. 1) — 70 points for motor function, 20 points for behavior, and 5 points each for visual field and facial movement. Animals that failed to survive received a score of zero. A score of 17 indicates a near moribund condition, 27 would indicate marked neurologic defi-

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*Endo Laboratories Inc., Garden City, N.Y., 11530.
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Results of neuropathologic evaluations are also presented in Tables 1 and 2. In the control group all animals had large infarcts off the MCA territory with scores of 80 with a range of 47 to 91. Nonparametric analysis of the results requires the use of “nonparametric” or more precisely “distribution-free” methods. At the recommendation of the Department of Biometry at CWRU, we used the Mann-Whitney Rank Sum Test (two-tailed).

We have not attempted to statistically analyze the results of the neurologic evaluations performed during the period of acute laboratory observation. These examinations were made by unblinded observers, and at the time of the experiments were intended only to provide a standardized method of recording neurologic function. Conclusions based on such an analysis would only be clouded by the possibility of bias.

Results

In all animals, marked right hemiparesis and right homonymous hemianopsia developed within seconds of inflating the balloon occluder; neurologic scores deteriorated to a range of 17 to 27 out of the possible 100.

Therapy with naloxone produced a modest improvement in ischemic deficits in five of the seven treatment animals during the period of occlusion (neurologic scores improved an average of 25 points.) Within minutes of administering the loading dose of naloxone, responding animals were more alert and again responded aggressively to touch. Motor function in the paretic extremities improved so that animals were once again capable of grasping when provoked, although strength remained decreased. No animal in the control group demonstrated such improvement in neurologic function during the same period of observation.

All five of the responding animals in the treatment group had an uneventful course following release of the occluder and went on to sustain only mild (three of the five animals) or moderate neurologic deficits when evaluated at 10 days. In contrast, the two animals that failed to respond to naloxone died secondary to progressively increasing ICP within 48 hours of releasing the occluder. Three of the seven animals in the control group likewise died of progressive malignant ICP following release of the occluder. Among the survivors in the control group, three of the four animals were near moribund at final evaluation while the remaining animal was moderately impaired.

Final neurologic scores for both groups of animals are presented in Tables 1 and 2. Although mortality in the two groups was similar, the neurologic outcome for animals surviving the occlusive episode was significantly better in those treated with naloxone. In the control group the median neurologic score after 10 days of observation was 22 with a range of 17 to 47. In the naloxone group the median score in surviving animals was 80 with a range of 47 to 91. Nonparametric analysis of these results using the Mann-Whitney Rank Sum Test (two-tailed) indicated that the differences are significant (p < 0.05).

Results of neuropathologic evaluations are also presented in Tables 1 and 2. In the control group all animals had large infarcts off the MCA territory with scores of 60 points. Because the raw calculated score is 38 percent, it is rounded to 40, and the animal would receive a neuropathologic score of 60 points.

These scores provide two independent means of assessing final outcome in each experimental group. Because the grading scale is not continuous over the range of 0–100 points in either of these evaluations, statistical analysis of the results requires the use of “nonparametric” or more precisely “distribution-free” methods.
scores of 40 or less (median 20). In the naloxone group, however, three animals received neuropathologic scores of 90. In these three animals, therapy with naloxone almost totally prevented ischemic tissue injury despite six hours of MCA occlusion. While these results reveal a clear trend for less severe injury in the treatment group (Table 2), statistical analysis failed to demonstrate significance at the accepted 0.05 level ($p = 0.056$, Mann-Whitney Rank Sum Test — two-tailed.)

The mean arterial pressure and cardiac output data are presented in Table 3. Neither occlusion nor therapy with naloxone significantly affected these variables.

### Discussion

Controversy continues to surround the opiate antagonist naloxone. Recent work has been presented both supporting and contradicting claims that it reverses ischemic neurologic deficits. 6,7,8,9,10

Frequent failure of naloxone to reverse the neurologic deficits of cerebral infarction, particularly late stable deficits, is not unexpected.

To test the hypothesis that naloxone reverses ischemic neurologic deficits (or that it has a protective effect during temporary focal cerebral ischemia), it must be given before the onset of irreversible cellular damage. When ischemia is the result of occlusion of a major cerebral vessel, the timing of therapy becomes the major critical factor.

### Timing of Therapy

Irreversible cell damage is not an immediate result of the focal cerebral ischemia that follows MCA occlusion. In our model, Spetzler, et al. previously reported that animals subjected to 10 minutes of MCA occlusion rapidly recovered without neurologic or pathologic evidence of infarction. 6 Based on evoked potential responses in the baboon, Branston and co-workers similarly have reported that even in cortical areas where CBF is maximally depressed after MCA occlusion, complete recovery of functional electrical activity can be expected if reperfusion is established within 15 minutes. 12, 13

Most recently, Jones et al. described the results of varying intervals of MCA occlusion in awake monkeys. 14 They reported that careful pathologic evaluation revealed only microscopic foci of infarction after 15 to 30 minutes of ischemia, while moderate to large infarcts were present after 2 to 3 hours of ischemia, and large infarcts occurred in most cases of permanent MCA occlusion.

Thus, despite the presence of marked functional impairment after MCA occlusion, it would appear that residual blood flow is sufficient to maintain the viability of the majority of cells for approximately one-half hour. To be maximally effective, then, therapeutic interventions after the occlusion of a major cerebral vessel must be initiated within this short grace period.

### Dosage

In models of hypovolemic and endotoxic shock, as well as spinal trauma, reports documenting beneficial effects for therapy with naloxone have involved dosages in the range of 5 to 10 mg/kg. 15-21 While this far exceeds the usual therapeutic dosage and may seem excessive, at least two reports indicate that results are dose dependent in these models and not maximal below 5 mg/kg. 18, 20 Therefore, we initiated therapy in this study with a dosage of 5 mg/kg.

Because of naloxone’s short half-life (approximately 20 minutes), to continue therapy the initial bolus was followed by a constant infusion of 5 mg/kg/hr. 22

### The Model

Oclusion of the MCA in the awake restrained baboon provides a unique controlled setting in which to evaluate the effect of therapy for focal cerebral ischemia. Animals are alert and remote from the complicating effects of anesthesia and surgery, and the clinical deficits and distribution of ischemia are similar to those observed in man. 23, 24

The reliability of the occluder used in this model has been previously demonstrated using standard angiographic techniques. 6 The rapid development of neurologic deficits following MCA occlusion documents the onset of ischemia in each animal. Animals that fail to develop the expected deficits following inflation of the occluder are rigorously excluded from further study (1 of 15 in the present study). To further insure proper function of the occluder, a radio-opaque contrast is used as the hydraulic inflation medium in the assembly. Lateral skull films taken just before and after release of the occluder can then be used to document

### Table 1

**Neurologic and Neuropathologic Outcome for All Animals**

<table>
<thead>
<tr>
<th>Controls</th>
<th>Neurological Score</th>
<th>Pathological Score</th>
<th>Naloxone</th>
<th>Neurological Score</th>
<th>Pathological Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>17</td>
<td>10</td>
<td>1</td>
<td>80</td>
<td>90</td>
</tr>
<tr>
<td>2</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>50</td>
<td>30</td>
</tr>
<tr>
<td>3</td>
<td>27</td>
<td>20</td>
<td>3</td>
<td>0</td>
<td>0</td>
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<tr>
<td>4</td>
<td>0</td>
<td>0</td>
<td>4</td>
<td>47</td>
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<tr>
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<td>0</td>
<td>0</td>
<td>7</td>
<td>80</td>
<td>90</td>
</tr>
</tbody>
</table>

Neurologic scores of zero indicate that the animal died prior to final neurologic evaluation (ten days after the occlusive episode). Neuropathologic scores of zero indicate that death was due to massive swelling and herniation of the ischemic hemisphere.

### Table 2

**Final Median Neurologic and Neuropathologic Scores for the Survivors in Both the Control and Treatment Groups (p-values Calculated Using the Two-tailed, Mann-Whitney Rank Sum Test)**

<table>
<thead>
<tr>
<th></th>
<th>Survivors</th>
<th>Controls</th>
<th>Naloxone</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neurologic score (median)</td>
<td>22</td>
<td>80</td>
<td></td>
<td>0.035</td>
</tr>
<tr>
<td>Pathologic score (median)</td>
<td>20</td>
<td>90</td>
<td></td>
<td>0.056</td>
</tr>
</tbody>
</table>
that the balloon remained fully inflated throughout the occlusion and to insure that it is fully deflated after release.

Neurologic Evaluation

Our scoring system for neurologic evaluation was designed to stratify animals on the basis of neurologic manifestations after MCA occlusion. Thus, motor function accounts for 70% of the possible score, and the presence of even a mild hemiparesis indicates a major deficit with respect to the MCA territory (Fig. 1). Considering the primary role of this area in the control of motor function, weighting of the neurologic evaluation in this manner seems appropriate. Furthermore, correlation of final neurologic scores with the findings on pathologic evaluation in this study demonstrates that the results of neurologic evaluation provide an accurate assessment of the amount of cerebral damage that can be expected (Table 1).

Response to Naloxone

In our model, the administration of naloxone one-half hour after MCA occlusion produced a modest improvement in ischemic neurologic deficits in five of the seven treatment animals. Within minutes of receiving the initial bolus, animals were more alert and again responded aggressively to stimulation of the paretic extremity. No animal, however, had a complete recovery of function. In our model, then, naloxone did not totally reverse ischemic deficits as previously described by Baskin and Hosobuchi.1 We feel that this variation in results was secondary to differences in the severity of ischemia. Evidence from the recent literature suggests that during focal cerebral ischemia, areas of the brain may remain functionally silent yet viable and capable of returning to normal activity.12-13, 25, 26

In their clinical report, Baskin and Hosobuchi described two patients that responded dramatically to naloxone with a complete reversal of ischemic deficits.1 In both patients, deficits followed neurosurgical procedures that did not involve occlusion of a major cerebral vessel. Despite the presence of stable ischemic deficits, CT scans in both patients showed no evidence of infarction. In these two patients, then, focal cerebral blood flow was reduced sufficiently to impair functional neurologic activity but not sufficiently to produce infarction. The large infarcts sustained by all animals in our control group certainly indicate that they suffered a much more severe ischemic insult than the described patients.

Holaday and D'Amato have suggested that the early improvement in neurologic function in some models may be due to the stimulation produced by handling.27 In gerbil models of cerebral ischemia these authors reported that simple handling of neurologically impaired gerbils resulted in a decrease in apparent ischemic deficits within 3 to 5 minutes and that the effect lasted 15 to 30 minutes.4 Holaday has suggested that these effects might inadvertently be attributed to naloxone therapy. Following MCA occlusion in our model, all animals received a minimum of three repeat neurologic exams prior to the administration of naloxone therapy. Improvement secondary to handling and arousal were not observed during this 30 minute period. Improvements, however, were apparent in five of the seven animals within minutes of receiving the initial bolus of naloxone. In our model, then, naloxone and not the stimulation of handling alone appears responsible for these early improvements in neurologic function.

More therapeutically important than the partial reversal of ischemic deficits, however, is the finding that therapy with naloxone (as described) appears to have had a protective effect during temporary focal cerebral ischemia. When results were compared for both groups of survivors, treatment with naloxone was found to improve final neurologic scores while ameliorating ischemic tissue injury. Results stand out in three animals in particular. In these three, therapy with naloxone almost totally prevented ischemic tissue injury. Final neurologic scores for these animals ranged from 80 to 90, and pathologic evaluation revealed only small focal areas of infarction and pallor (neuropathologic scores of 90 in all three).

Based on the total experience of this laboratory with over 100 primates, only barbiturate induced coma at 30
minutes, as reported by Selman et al.28 produced consistently better results following a six-hour period of MCA occlusion.

While these results are encouraging, we must stress that the response varied from animal to animal, and that when compared statistically only the differences in neurologic scores among survivors were significantly valid at the 0.05 level (Table 2). When results were compared for both groups overall (survivors and non-survivors) there were no significant differences in outcome. This apparent inconsistency arises as a result of the overlap produced by neurologic and neuropathologic scores of zero in both groups for those animals dying secondary to massive edema and brain swelling after the ischemic insult. Thus, therapy with naloxone did not effect mortality in this model, but did appear to reduce overall morbidity among survivors.

Mechanism

Beginning in 1978 Holaday and Faden, and then numerous other groups, documented the effectiveness of naloxone in the management of hemorrhagic and endotoxic shock.13-16,18-20 Naloxone has also been shown to improve neurologic recovery following spinal contusion.17,21 Improvements in outcome for animals treated with naloxone in many of these studies have been related to increases in cardiovascular performance. No such correlation was apparent in the present study when mean arterial pressure and cardiac output data were analyzed for differences both between the two groups, and within the naloxone group before and after the initiation of therapy.

It would seem reasonable to assume that while all animals developed marked hemiparesis and hemianopia following MCA occlusion, those surviving the period of ischemia (both in the control and treatment groups) suffered a less severe insult. In this regard, Branston and coworkers have demonstrated the existence of separate ischemic thresholds for the loss of functional electrical activity and cellular integrity.12,13,25,26 Electrical activity ceases at approximately 15 ml/100 g/min, while energy failure with impending cell death (indicated by the massive release of intracellular potassium) does not occur until blood flow drops below approximately 8 ml/100 g/min. The findings in these reports suggest that small variations in CBF may markedly affect the outcome of an ischemic episode. Studies are planned to examine the effect of naloxone on local CBF in our primate model using radio-labeled microspheres.

The pharmacologic properties of naloxone are well characterized.22,29 It is a potent, highly specific opiate antagonist that in the narcotic naive "normal" subject appears nearly devoid of pharmacologic activity even at markedly elevated dosages.30,31 Because of its highly specific nature, therapeutic responses to naloxone have always implied the clinical involvement of opiate antagonists. The obvious implication is that in the absence of exogenous opiates naloxone must produce its therapeutic benefits by antagonizing the endorphins. However, until more is known about the cellular and regulatory roles of the opiate peptides, this will remain conjecture.

Conclusion

Evaluation of naloxone's role in the therapy of focal cerebral ischemia requires careful consideration of both the timing of therapy (with respect to potential cellular viability) and the dosage of drug.

Results in our primate model confirm earlier reports that naloxone is capable of at least partially reversing ischemic deficits secondary to focal cerebral ischemia. More importantly, our results suggest that therapy with naloxone may have a protective effect during temporary focal cerebral ischemia. Certainly further studies are indicated.

Acknowledgments

Special thanks to Endo Laboratories (Endo Laboratories, Inc., Garden City, N.Y., 11530) for supplying crystalline naloxone for use in this experimental trial.

References

A Double Blind Trial of Naloxone in the Treatment of Acute Stroke

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SUMMARY Naloxone has been reported to have potential benefit in the treatment of stroke. We evaluated the effect of naloxone in a double-blind trial conducted with 15 stroke patients whose deficits ranged from 8 to 60 hours in duration. All but one patient sustained a cerebral infarction. Neurologic function was assessed before and five minutes after each of two injections given to each patient in a double-blind fashion. The injections consisted of naloxone (0.4 mg in 3 patients and 4.0 mg in 12 patients) and saline. Prior to the trial, samples of plasma were obtained for determination of immunoreactive beta-endorphin for each patient. Four patients showed minimal improvement following injection of naloxone, while five patients exhibited a slightly greater improvement following saline injection. There were no significant elevations of plasma beta-endorphin among stroke patients. We conclude that naloxone may not have a significant therapeutic role for stroke in the clinical setting.

THE ENDOGENOUS OPIATE SYSTEM may play a role in the pathogenesis of a number of disease entities, including spinal cord contusion1 and a variety of shock syndromes.2 Recently, the opiate antagonist naloxone has been used to treat cerebral ischemia in both limited clinical trials1 and in animal models of stroke.1,3 Naloxone was initially reported to be of benefit in human cerebral ischemia,4 but further studies, using the drug in doses up to 1.2 mg, have produced conflicting results.5,6 In an attempt to define the clinical efficacy of naloxone, we undertook a prospective, double-blind trial of this agent at doses of up to ten times those initially reported to be of benefit in acute stroke patients.

Methods

Patients admitted to Los Angeles County University of Southern California Medical Center with stroke of less than 72 hours in duration were studied after informed consent was obtained. Exclusion criteria consisted of pregnancy, use of narcotic drugs, shock, spinal cord injury and other recent trauma. All patients received head CT scans. The neurological function of each patient was scored according to a standardized examination immediately before and five minutes after each of two injections given one hour apart in a double-blind fashion. The injections consisted of naloxone and normal saline. The first three patients in the study received 0.4 mg naloxone and the remaining subjects received 4.0 mg. Blood pressures were monitored.
Naloxone therapy during focal cerebral ischemia evaluation in a primate model.
J M Zabramski, R F Spetzler, W R Selman, U R Roessmann, L A Hershey, R C Crumrine and R Macko

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