Ischemic neuronal damage after acute subdural hematoma in the rat: effects of pretreatment with a glutamate antagonist

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The ability of a competitive N-methyl-D-aspartate (NMDA) receptor antagonist (D-CPP-ene) to reduce irreversible brain damage has been examined in a rodent model of acute subdural hematoma. Acute subdural hematoma was produced by the slow injection of 400 μl homologous blood into the subdural space overlying the parietal cortex in halothane-anesthetized rats. Brain damage was assessed histologically in sections at multiple coronal planes in animals sacrificed 4 hours after induction of the subdural hematoma. Pretreatment with D-CPP-ene (15 mg/kg) significantly reduced the volume of ischemic brain damage produced by the subdural hematoma from 62 ± 8 cu mm (mean ± standard error of the mean) in vehicle-treated control rats to 29 ± 7 cu mm in drug-treated animals. These data demonstrate the anti-ischemic efficacy of NMDA antagonists in an animal model of intracranial hemorrhage in which intracranial pressure is elevated, and suggest that excitotoxic mechanisms (which are susceptible to antagonism by D-CPP-ene) may play a role in the ischemic brain damage which is observed in patients who die after acute subdural hematoma.

KEY WORDS • subdural hematoma • ischemic brain damage • neuronal damage • glutamate antagonist • rat

Recent advances in the treatment of brain ischemia have included the identification of several therapeutic agents which are highly effective in the laboratory. Among these are calcium entry blockers, free radical quenchers such as lazaroïds, and excitatory amino acid antagonists. Calcium antagonists, furthermore, have clearly shown clinical efficacy and safety in the specialized role of prophylactic pretreatment after subarachnoid hemorrhage, although they are less effective than other agents in the laboratory. Of the newer “neuroprotective” compounds, glutamate antagonists are currently a major focus of interest for neurosurgeons. Their efficacy in reducing damage due to focal ischemia exceeds that of other agents, and they have been shown to reduce neurological dysfunction after traumatic injury, both in the brain and spinal cord.

Unfortunately, major side effects associated with some of these compounds have recently become apparent, which means that their use must be tempered with caution. The challenge facing clinicians who care for patients at risk for ischemic brain damage is, therefore, to identify those patients in whom there is an acceptable balance of risk versus likely benefit from such compounds. Patients with acute subdural hematoma may fall into this category. At least 50% of patients who undergo surgical removal of an acute subdural hematoma will die and persisting neurological disability will affect 25% to 35% of those who survive.

Ischemic brain damage is the commonest neuro-pathological abnormality found in patients who die of acute subdural hematoma. This is distributed chiefly in the hemisphere ipsilateral to the hematoma, although it may be present in both hemispheres when intracranial pressure (ICP) is severely raised. Once a subdural hematoma has been removed, the inadequacy of therapeutic options which are available to control ICP and limit ongoing neurological damage means that these patients often present the neurosurgeon with particular difficulties in management. For these reasons, glutamate antagonists may become a viable treatment modality in these patients.

We have previously reported a model of acute subdural hematoma in the rat, in which a zone of acute cortical ischemic damage, involving 14% to 16% of the
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FIG. 1. Diagrams after subdural hematoma in two animals showing the extent and distribution of ischemic brain damage (black areas) at eight predetermined stereotactic planes. Left: Ischemic damage in a control animal (98 cu mm total ischemic volume). Right: Ischemic damage in a D-CPP-ene-pretreated animal (22 cu mm total ischemic volume).

volume of the hemisphere develops under the hematoma. In this report we have used the same model to evaluate the effects of pretreatment with a newly synthesized competitive N-methyl-D-aspartate (NMDA) antagonist, D-CPP-ene (E)-4-(3-phosphonoprop-2-enyl) piperazine-2-carboxylic acid (CPP-ene).2

Materials and Methods

These animal studies were performed under license from the Home Office, Scotland. Eighteen adult male Sprague-Dawley rats, each weighing from 362 to 530 gm, were studied. The animals were randomly allocated to the drug-treated or control group and were kept anesthetized throughout the experiments, with key physiological variables monitored and controlled.

General Preparation

The animals were placed in a Perspex chamber and anesthetized using a mixture of nitrous oxide and oxygen (70%/30%) and 2% halothane. Tracheostomy was performed and the animals were ventilated to normocarbia using a small-animal ventilator. The right femoral artery and femoral vein were cannulated for continuous arterial pressure monitoring and administration of drugs and fluids, respectively. Body temperature was maintained at 37°C by means of a rectal thermometer and a heater system. After surgical procedures, halothane concentrations were kept at 0.5% in both groups. Blood gas analysis was performed before induction of the hematoma, 10 minutes after induction, and at hourly intervals thereafter, until perfusion-fixation at 4 hours.

Administration of D-CPP-ene or Placebo

For infusion, D-CPP-ene was freshly made up with 0.9% saline at a concentration of 1 mg/ml of solution, and administered to the drug-treated group at a dose of 15 mg/kg body weight. The infusion was commenced 15 minutes prior to induction of the acute subdural hematoma, and given over 10 minutes. In the control group, the same volume of 0.9% saline alone was in-
fused. At the end of the experiment, venous blood was sampled for drug level determination.

**Induction of Acute Subdural Hematoma**

Following general preparation, the animals were positioned prone and a midline scalp incision was made after infiltration with 1% lidocaine. The bregma was exposed and a 3-mm burr hole was drilled 3 mm lateral to the sagittal suture and 1 mm posterior to the coronal suture. Under an operating microscope, the dura was incised and a J-shaped No. 23 blunt-tipped needle was placed into the subdural space. The needle was cemented into position using rapid-setting cyanoacrylate glue. Next, 0.4 ml of nonheparinized homologous venous blood was drawn and injected into the subdural space over a period of 7 minutes. At the end of the injection procedure, the subdural needle was sealed.

**Quantitation of Ischemic Neuronal Damage**

The animals were allowed to survive under controlled ventilation for a 4-hour period, and intermittent blood gas analysis and continuous arterial pressure monitoring were carried out. At the end of the survival period, the animals were turned supine and anesthesia was deepened by increasing the halothane concentration. A midline thoracotomy was performed and the heart and great vessels were exposed. A No. 14 cannula was inserted through the left ventricle into the aorta, and the right atrium was incised while the animal was perfused with 100 ml of heparinized saline at mean arterial pressure. When the perfusate was clear, the animals were perfusion-fixed with 200 ml of formaldehyde/glacial acetic acid/methanol (FAM) (1:1:8, v:v). The heads were stored in FAM for at least 24 hours. The brains were removed from the skull, the left cerebral hemisphere was identified with indelible ink, the hindbrain was detached at the level of the cerebellum, and the forebrain was cut into four equally spaced coronal slices. These were then dehydrated through graded ethanols and embedded in wax. Histological sections 7 μ thick (about 100 sections per brain) were cut throughout the forebrain and stained with hematoxylin and eosin or cresyl violet and Luxol fast blue. Sections corresponding to eight predetermined stereotactic planes distributed throughout the forebrain were identified and examined under both high- and low-power light microscopy. The extent of ischemic damage was defined in each brain section and annotated onto enlarged line diagrams of the rat brain based on the atlas of Koenig and Klippel (Fig. 1). The quantity of ischemic damage at the eight predetermined stereotactic planes studied was then measured with an image-analyzing computer,* and the volume of ischemic damage

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*Quantimet computer, Model 970, manufactured by Cambridge Instruments, Cambridge, England.
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was calculated by integrating the area under the curve so obtained, according to the method of Osborne, et al.21 (Fig. 2). Histological quantification was performed by a neuropathologist (D.I.G.) without knowledge of the drug/placebo randomization.

Results

Mean arterial blood pressure and arterial blood gas values for the control and D-CPP-ene-treated groups are shown in Fig. 3. There were no significant differences in arterial blood pressure between the control group and those pretreated with D-CPP-ene. The infusion of D-CPP-ene in a relatively large volume of saline over 10 minutes caused no significant changes in blood pressure. A mild Cushing’s response was noted in both the D-CPP-ene-treated and control animals. Normocarbia and normothermia were maintained in all animals (Fig. 3). Arterial oxygenation was similar in both groups immediately prior to the hematoma (mean ± standard error of the mean: 183 ± 19 mm Hg in the control group and 183 ± 16 mm Hg in the D-CPP-ene-treated group), and there were no significant differences from these levels or between groups throughout the experiment.

Blood Levels of D-CPP-ene

Levels of D-CPP-ene were measured by a high-performance liquid chromatography technique in the pretreated animals prior to perfusion-fixation. The mean plasma level was 2.68 µg/ml. In a single-compartment kinetic model to predict plasma clearance of CPP-ene, $C_t = C_\infty e^{-\frac{t}{\tau}}$ where $C_t$ is the plasma concentration at time $t$, $C_\infty$ is the peak plasma level, $e$ is the natural logarithm or exponential function, and $K_e$ is the elimination constant or plasma clearance of CPP-ene. For D-CPP-ene, $K_e$ is 0.75, $t$ is 4 hours, and $C_\infty$ is calculated to be around 50 µg/ml.

Distribution and Nature of Acute Subdural Hematoma

In all the animals studied, subdural clots were visible overlying the hemisphere when the brain was removed (Fig. 4). Because of shrinkage due to FAM fixation, the hematomas were less extensive than those that we have seen when unfixed brains are removed in this model.20 The hematomas were variable in both size and situation over the hemisphere despite the constant volume of blood that was injected. In one animal in the D-CPP-ene-treated group, the hematoma was seen to extend onto the opposite hemisphere and into interhemispheric fissure. In two of the D-CPP-ene-treated animals and in four of the control group, small intracerebral hematomas were found coexisting with the acute subdural hematoma but these did not exceed 1.5 mm in diameter, and none was a significant space-occupying lesion in itself.

Distribution and Nature of Ischemic Brain Damage

All brains were well perfusion-fixed: intravascular blood was absent and dark cell or hydropic change artifacts were not seen.18 Ischemic brain damage was present in all animals in the control and D-CPP-ene-treated groups, but was slight in some of the D-CPP-ene-treated animals. In all animals, ischemic neuronal damage was found only in relation to the overlying subdural blood clot and ischemic damage was most marked where the overlying clot was thickest (Fig. 4). In some areas, only a thin film of blood clot was present, overlying fairly extensive ischemic change in the underlying cortex. The area of ischemic damage was sharply

Fig. 4. Low-power photomicrograph sections through the rat brain at the level of the caudate nucleus (left) and hippocampus (right) 4 hours after subdural hematoma. Note the zone of pallor of staining and ischemic change, well demarcated from normal brain, which is present beneath the subdural hematoma. H & E, × 10.
delineated from normal brain by a clear-cut boundary (Fig. 4). The features seen in the areas with early infarction did not differ from those seen in other animal models of acute ischemia, such as middle cerebral artery occlusion, and these included microvacuolation and shrinkage of neuropil, pyknosis and hyperchromasia of the neuronal bodies and nuclei, and swelling of perineuronal astrocytes.\(^{2,25,27}\) In three control group animals with large lesions, a thin rim of ischemic neuronal damage was seen to involve the outer edge of the caudate nucleus but this was not seen in any of the D-CPP-ene-treated animals.

Our results showed that pretreatment with D-CPP-ene effected a marked reduction in the volume of ischemic brain damage from the subdural hematoma (62.1 ± 7.9 cu mm in the control animals vs. 28.7 ± 6.5 cu mm in the D-CPP-ene-treated animals; \(p = 0.019\), Mann-Whitney test, Fig. 5). It also reduced the area of ischemic damage in most stereotactic planes examined. The most consistent and marked changes were noted in the planes where damage was greatest in the control animals (immediately adjacent to the hematoma).

**Discussion**

**Glutamatergic Neuronal Injury**

Excessive concentrations of excitatory neurotransmitter substances acting upon postsynaptic receptors may cause prolonged depolarization of ion channels, with consequent potassium ion efflux, and sodium and calcium influx into neurons.\(^{29,31,35}\) This process may lead to damage of neuronal cytoplasmic enzyme and second-messenger systems, and subsequent neuronal vacuolation and pyknosis, together with massive K\(^+\)-induced astrocytic swelling.\(^{30,29,35}\) This sequence of events was first described by Olney, et al.,\(^{35}\) after studies in which supraphysiological plasma concentrations of glutamate were shown to cause neuronal damage in areas of the immature mouse brain, with an open blood-brain barrier.

The ultrastructural and light microscopic characteristics of glutamate-induced neuronal damage are indistinguishable from those of early ischemic neuronal damage; moreover, the distribution density of glutamatergic receptors within the brain closely corresponds to the anatomic hierarchy of selective vulnerability to global ischemic damage.\(^{2,21}\) This led to speculation that a glutamatergic mechanism may be partly responsible for mediating neuronal damage in cerebral ischemia.\(^{20,21,24}\) Support for this hypothesis has been provided by the demonstration of massive increases (up to 20-fold) in extracellular glutamate levels using microdialysis techniques in circumstances of both global and focal cerebral ischemia.\(^{6,14}\)

**Glutamatergic Receptor Subtypes**

Three postsynaptic glutamatergic receptor subtypes have now been identified and characterized, and their distribution in various brain regions has been studied.\(^{21,20,35}\) The NMDA receptor represents about 70% of glutamatergic receptors, and is present in particularly high concentrations in the hippocampus and cortex.\(^{31,29,35}\) Several specific antagonists for this receptor have now been evaluated. This is known of these antagonists, MK801, has been shown to be highly effective in protecting against the in vivo neurotoxicity of glutamate, and its analog NMDA.\(^{19,26,37,35}\) Subsequently, many studies have confirmed this neuroprotective effect. This has been most convincing in models of focal cerebral ischemia, such as middle cerebral artery occlusion, in both the cat and rat.\(^{26,27}\) Moreover, MK801 has been shown to reduce the extent of focal ischemia in cats after middle cerebral artery occlusion even when given 2 hours after the ischemic insult.\(^{27}\)

**Noncompetitive NMDA Antagonists**

Noncompetitive NMDA antagonists, for example, MK801, ketamine, phencyclidine, and magnesium ions, are maximally effective in blocking agonist-operated ion channels in circumstances when glutamate concentrations are high, indicating that their blockade is use-dependent.\(^{15,30}\) Although this use-dependent blockade is theoretically advantageous in circumstances of ischemia where glutamate concentrations are high, the high receptor affinity and nondisplaceable binding characteristics of MK801 indicate that its effects upon the ion channel are prolonged and cannot be overcome by physiological concentrations of presynaptic glutamate within normal brain regions, unaffected by ischemia. This means that noncompetitive NMDA antagonists cause profound functional derangement in areas of the normal brain where neurotransmission is mediated by glutamate.\(^{27}\) Some of these agents may thus cause prolonged and severe behavioral and motor disturbances such as catatonia, ataxia, decreased consciousness, and repetitive stereotyped movements; in man, these agents may be psychotomimetic.\(^{17,24}\) These same characteristics have led to prolonged limbic hyperstimulation, most evident in the rat as glucose hy-

![Fig. 5. Mean volume ± standard errors of the means) of ischemic damage after subdural hematoma in eight control rats and 10 D-CPP-ene-pretreated rats. The difference was significant (\(p < 0.019\), Mann-Whitney test, asterisk).](image-url)
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permetabolism in the posterior cingulate cortex, hippocampus, and mamillary bodies. Recently, delayed cytoplasmic structural damage to cingulate neurons, which appears to be reversible, has been demonstrated after MK801 and phencyclidine administration in normal rats.

Competitive NMDA Antagonists

Competitive NMDA antagonists, by contrast, are not use-dependent and may be displaced from the NMDA receptor site by physiological concentrations of neurotransmitter glutamate, so theoretically these agents should be associated with less crenagement of normal brain function. The newly synthesized competitive NMDA antagonist, D-CPP-ene, is one of the most potent antagonists for the NMDA receptor site, although it is less lipophilic than MK801. Preliminary studies indicate that this agent reduces the extent of hemispheric ischemic damage by 65% in the feline middle cerebral artery occlusion model: a level of neuroprotective efficacy comparable to MK801. Unfortunately, competitive NMDA antagonists have not shown neuroprotective efficacy when administered more than 5 minutes after the ischemic event, in contrast to MK801; thus, in clinical use, these safer compounds may be most valuable when they can be given prophylactically, before an ischemic event.

NMDA Antagonists and Ischemic Damage After Acute Subdural Hematoma

The purpose of these studies has been to test whether an NMDA antagonist is capable of reducing the cerebral ischemia which accompanies acute subdural hematoma. We have chosen to pretreat the animals in the D-CPP-ene-treated group, in order to provide optimal drug delivery to the ischemic cerebral tissue. Pretreatment with D-CPP-ene is associated with a 54% reduction in the extent of ischemia in these studies. This accords closely with similar results of studies using MK801 in the rat and the cat middle cerebral artery occlusion models and with our results using D-CPP-ene in the cat middle cerebral artery occlusion model.

Although the pattern of ischemic damage seen in this rat model of acute subdural hematoma is similar to the focal hemisphere ischemic damage seen in humans who die after acute subdural hematoma, its origin is poorly understood. It seems unlikely that local mass effect and consequent compression of the microcirculation are responsible; in our study, ischemic damage was seen underlying even thin films of blood over the cortex, without distortion of the underlying brain in these animals (Fig. 4). It seems likely that the presence of the subdural blood may initiate release of substances toxic to neural tissue. We have shown, using microdialysis techniques, that the acute subdural hematoma is associated with a massive but transient release of glutamate (to six times normal levels for 20 minutes) in the underlying cortex. Studies of local cerebral blood flow by autoradiographic techniques in this model have also demonstrated profoundly reduced cerebral blood flow in the cortex underlying the hematoma.

Conclusions

It appears that NMDA antagonists may exert their neuroprotective effects in this model by preventing glutamate-induced neuronal damage within the cortical ischemic zone. This study is the first to demonstrate the efficacy of a glutamate antagonist in this pathophysiological setting. Further studies are needed to assess whether treatment begun after the hematoma has been initiated will be effective. Noncompetitive NMDA antagonists, which exhibit posttreatment efficacy when given 2 hours after the ischemic event, may be preferred for these studies. The present investigation provides a basis for trials to evaluate the effects of NMDA antagonists in humans at risk for brain damage due to posttraumatic intracranial hematomas.

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