

TECHNICAL NOTE: PRELIMINARY RESULTS IN DEVELOPMENT OF A NOVEL INTRACISTERNAL PENICILLIN SEIZURE MODEL IN THE RAT

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1. ABSTRACT

In order to develop an intracisternal penicillin rat model of epilepsy, eleven anesthetized male Wistar rats were studied. 5 underwent intracisternal injection of penicillin (doses 150,000-300,000 units) in the prone position, and another 5 underwent intraperitoneal penicillin injection; one died following intracisternal injection, prior to further study. Time between penicillin injection and seizure induction (determined by electroencephalography) was recorded. Each animal had a tracheostomy, and was mechanically ventilated and carefully monitored for adverse effects. Seizures were noted in an average of 13:42 minutes following penicillin injection (range 4:30-23:20) for the intracisternally (IC) injected group. Both episodic and continuous seizure activity was seen, and a dose-dependent effect was seen (quicker-onset, more continuous seizures with higher doses, in the IC group). Onset was significantly faster in the IC than for the intraperitoneally injected group (all >1 hour for the latter group in our study). 96 total separate seizure episodes were seen, ranging from 3 to 540 seconds. Epileptic activity could be seen in all IC-injected rats lasting over 1 hour into the study. The intracisternal penicillin injection rat model appears to provide a quick-onset, reliable method of inducing seizure activity in the rat model while leaving the cranial vault intact.

2. INTRODUCTION

Seizure generation models in animals have involved multiple modalities of seizure induction, in a variety of species (1). As comprehensively summarized by Fisher, over models were reviewed. Agents used have included topically applied convulsants, electrical stimulation, injury, kainic acid, and toxins. Preparations have included hippocampal slices, and isolated cell preparations. Species analyzed have included genetically seizure-prone strains of mouse, rat, gerbil, fruitfly, and baboon, amongst others. Specific targeted brain stimulation has also been evaluated for seizure induction, including thalamic and bilateral cortical stimulation.

The use of penicillin as an epileptogenic material was discovered during its use as part of an irrigant for neurosurgical procedures. Patients were noted to have seizures, and after it became apparent that penicillin was implicated, this practice was stopped. Topical use of penicillin has been shown to be an effective method of seizure induction. If the cranial vault is to be maintained intact, however, the alternative method of intracisternal penicillin injection can be explored. While intraperitoneal, intravenous, and direct cortically administration of penicillin in rats have been described, we have not found a single report of intracisternally administered penicillin in

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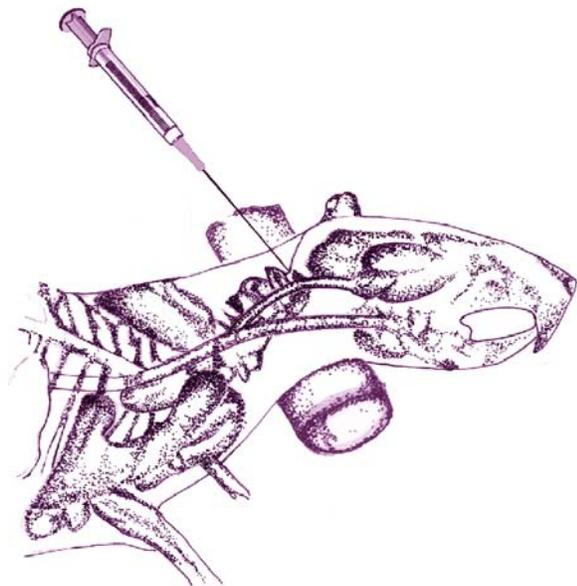


Figure 1. Method of intracisternal penicillin injection in the rat.

the rat (2). This method may offer the advantage of quicker-onset seizures while maintaining the integrity of the cranial vault. The efficacy, apparent side effects, and technique of this model is described here, in preparation for testing electroconvulsive therapy as a seizure control method (which is described in a subsequent paper).

3. MATERIALS AND METHODS

Approval for this study was obtained from the Animal Use Committee at the Louisiana State University, Shreveport, Health Sciences Center. Eleven anesthetized male Wistar rats total were studied, with the plan of having five rats in each group completing the study. Intracisternal penicillin for injection was prepared by mixing 5 million units of Penicillin G in 5 cc of sterile water. After anesthetizing a rat intraperitoneally using a mixture of alpha-chloralose and urethane and performance of a tracheostomy (which placed the rat on a ventilator), a 27-gauge bent needle was passed through the exposed occipitoatlantal membrane and cerebrospinal fluid was in a rat anesthetized intraperitoneally using a mixture of alpha-chloralose and urethane. Rats weighed 200 \pm 50 grams; of these, one died following intracisternal administration, and ten were properly studied – 5 underwent intracisternal injection of penicillin (doses ranging from 150,000-300,000 units) in the prone position, and another 5 underwent intraperitoneal penicillin injection. The time between penicillin injection and seizure induction was recorded by electroencephalography. Seizure recording, or electroencephalography (EEG), was conducted using needle electrodes (A.D. Instruments, Colorado, U.S.A.) placed into the scalp, and has relatively quick onset according to our work.

Each animal had a tracheostomy performed prior to the procedure, and was monitored carefully for adverse

effects using blood pressure (via a femorally placed arterial line), heart rate, and respiratory changes. Animals were sacrificed at the end of approximately two hours of seizure recording.

The method of intracisternal penicillin injection in the rat is detailed in Figure 1.

4. RESULTS AND DISCUSSION

Seizures were noted in an average of 13 minutes, 42 seconds following penicillin injection (range 4:30 to 23:20) for the intracisternally (IC) injected group. Both episodic and continuous seizure activity was seen, and a dose-dependent effect was seen (quicker-onset, more continuous seizures with higher doses, in the IC group). Onset was significantly faster in the IC than for the intraperitoneally injected group (all greater than 1 hour in our study). 96 total separate seizure episodes were seen, ranging from 3 to 540 seconds. Epileptic activity could be seen in all IC-injected rats lasting over 1 hour into the study. Generalized-appearing seizures were noted each time, with EEG correspondingly noting a consistent pattern.

4.1. Seizure model

A multitude of seizure models have been described for studying the various different types of epilepsy, as extensively reviewed by Fisher(1). However, in a rat model, only the intraperitoneal (and cortical) methods of penicillin use were described as methods of inducing seizures. As our goal, it was imperative that the cranial vault was kept intact; also, a quicker onset than the intraperitoneal model was desired(2). Hence, the intracisternal model, previously described for other species, was attempted.

The mode of action of penicillin-induced seizure induction has been better elucidated more recently. In microdialysis studies *in vivo*, Shen et al found hippocampal increase in excitatory amino acids following chemical application of penicillin(3). Another study has suggested a phenomenon of "spreading depression" associated with epilepsy following penicillin application (4). Penicillin lowers the seizure threshold (5), as has been previously shown. While there is some inter-species variability of spike control using anti-epileptic medications in specifically penicillin-induced spikes (6), penicillin itself has become an extensively described model of seizure induction (particularly in intra-peritoneal route of administration)(1). The actual increase in cellular activity responsible for seizures has been noted in both cortical and sub-cortical levels, as increased cellular firing (above baseline) has been noted in both levels following penicillin-induced seizures (7). While thalamocortical circuits appear to be involved, it remains unclear whether both or one is involved in initiating such activity. Hyperpolarization and decreased inhibition have both been implicated in penicillin-induced epilepsy studies (8,9)

Other than establishment as an epileptogenic agent, the exact route of administration and dose of penicillin had to be elucidated for this study. One challenge

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was to maintain the intact integrity of the cranial vault, yet still administer intracranial penicillin; in strict conformance to this goal, stereotactic approaches were also precluded. Given the anatomy of the rat, an apparatus capable of causing intracortical administration via entry through the foramen magnum was not easily feasible. Hence, injection into the subarachnoid space accessed at the cisterna magna appeared a suitable alternative. While lumbar puncture was another consideration, flexion at the neck more readily facilitated injection through the posterior occipitoatlantal ligament. Intracisternal administration of drugs has been previously described in rabbits (10,11). Intraventricular injection of epileptogenic substances has been studied in cats (12). The ability to perform intracisternal injections has been shown in animals as small as mice, but for different indications (e.g. antibiotic injection)(13). As for rats, the animals studied in the present report, occasional reports of intracisternal puncture in rats for intracranial pressure recording (13), as well as lumbar puncture for delivery of drugs(14) have previously been described. However, the intracisternal penicillin model for seizure induction has not been described to date.

4.2. Advantages of Intracisternal vs. Intraperitoneal or Intravenous Use

As described, this model had the advantage of quick-onset, with long-lasting seizure occurrence. Seizures occurred on average in 13 minutes and 42 seconds (range 4:30 to 23:20). This is substantially faster than previous reports involving the intraperitoneal model, where spike activity was onset in 45.7 +/- 31.0 minutes (means +/- SD) and seizure activity within 71.5 +/- 38.4 minutes (2). The described qualities of maintaining an intact cranial vault, quick-onset seizures, and lasting seizures, allowed this model to be ideal for study of intervention using brain stimulation through an intact skull (a goal of future study in our laboratory).

Studies involving cerebrospinal fluid penetration of a penicillin analog have shown that less than 4% of the intravenous concentration is present in humans (16). In addition, the cerebrospinal fluid concentration of penicillin decreases as penicillin is actively absorbed (11).

4.3. Difficulties of Intracisternal vs. Intraperitoneal or Intravenous Use

Technically, the intracisternal space in the rat is small and requires some experience to access. This was facilitated in our studies by applying a gauze roll under the neck, which was flexed in the prone position (Figure 1). This opened the occipito-atlantal space, and access was gained by using a 30-gauge slightly bent Tuberculin syringe-needle. Upto 0.3 cc of cerebrospinal fluid was slowly removed, and then replaced with equivalent amount of 100,000 IU/0.1 cc. solution. The key step was to remain just lateral to the midline, so that brainstem/upper spinal cord injury could be avoided. The one casualty in our study, on post-mortem analysis, was likely related to injury to the brainstem-cervical spinal cord intersection from the needle traversing too medially.

5. SUMMARY

The intracisternal penicillin model provides the advantageous attributes of allowing quick-onset

generalized seizures, which persist in a recurrent fashion. Seizure onset is generally faster than intraperitoneal or intravenous models. Additionally, the cranial vault is maintained in intact fashion for specific experiments where this is desirable (such as extracranial stimulation). Technically, targeted methodology with experience can help optimize success.

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