Intraventricular and Intracerebral Delivery of Anti-epileptic Drugs in the Kindling Model

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Summary: A means to avoid the pharmacokinetic problems affecting the anti-epileptic drugs may be their direct intracerebroventricular (ICV) or intracerebral delivery. This approach may achieve a greater drug concentration at the epileptogenic area while minimizing it in other brain or systemic areas, and thus it could be an interesting therapeutic alternative in drug-resistant epilepsies. The objective of this article is to review a series of experiments, ranging from acute ICV injection to continuous intracerebral infusion of anti-epileptic drugs or grafting of neurotransmitter producing cells, in experimental models, especially in the kindling model of epilepsy in the rat.

Acute ICV injection of phenytoin, phenobarbital or carbamazepine is able to diminish the intensity of kindling seizures, but it is also associated with a high neurologic toxicity, especially phenobarbital. Continuous ICV infusion of anti-epileptic drugs can effectively control seizures, but neurologic toxicity is not improved compared with systemic delivery. However, systemic

toxicity may be improved, as in the case of valproic acid, whose continuous ICV infusion results in very low plasmatic or hepatic drug concentrations. Continuous intracerebral infusion at the epileptogenic area was studied as an alternative to minimize neurologic toxicity. Thus, intra-amygdalar infusion of gamma-aminobutyric acid (GABA) controls seizures with minimal neurotoxicity in amygdala-kindled rats. Similarly, continuous infusion of GABA into the dorsomedian nucleus of the thalamus improves seizure spread, while not affecting the local epileptogenic activity at the amygdala. Grafting of GABA releasing cells may reduce kindling parameter severity without behavioral side effects.

We may conclude that ICV or intracerebral delivery of anti-epileptic drugs or neurotransmitters may be a useful technique to modulate epilepsy. **Key Words:** Drug-resistant epilepsy, temporolimbic epilepsy, kindling model, intracerebroventricular route, intracerebral administration, neurotransmitter-releasing cells grafts.

INTRODUCTION

Antiepileptic drug (AED) resistance (i.e., the persistence of seizures despite proper administration of AEDs), constitutes a big clinical problem, affecting one third of epileptics.¹

To combat epilepsy, AEDs are given systemically (either through the oral or parenteral routes). However, whether located within a focus or distributed in a network, the epileptogenic area is localized in a limited area of the brain. The systemic route has two main drawbacks related to this fact. First, the pharmacokinetic processes of dissolve, absorption, distribution, metabolism, and excretion affect the drug outside its therapeutic compart-

ment. One of the main limitations of this process is the pass of the drug through the blood-brain barrier (BBB), which may be specifically affected in epileptogenic areas due to ischemia or gliosis.² This may be due to pharmacokinetic problems not specific to the drug, such as a decreased drug uptake into the brain by multidrug transporters in the blood-brain barrier.³ Second, systemic toxicity may limit the drug dosage. Toxicity limits the therapeutic interval of AEDs due to high drug concentrations in brain areas other than the target zone and may be one of the main causes of AED resistance.⁴

The previously cited limitations have led to the search of alternative strategies, including the delivery of the drug directly into the brain.^{4,5} The first approach may be the administration of drugs within the ventricular system or intracerebroventricular route (ICV). This route may show several advantages, due to its relatively easy access and the lack of need to specifically localize the epilep-

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togenic area. However, the cerebrospinal fluid is separated from the brain through the ependymal barrier whose properties are similar to the BBB. Besides, the main toxicity of AEDs is due to neurologic toxicity, which is due to the distribution of the drug to brain areas that may not be involved with epileptogenic activity.

These considerations can lead to a different approach: the direct infusion of AEDs within the brain, thus limiting its diffusion to the potential epileptogenic areas.

This can be achieved with the infusion of the drug using diffusion or convection forces, through a catheter, by a slow drug-releasing substance implanted into the brain, or even implanting cells, which may secrete the appropriate substance locally.

The development of this approach in epilepsy has been done in several experimental models, especially the kindling model.

THE KINDLING MODEL OF EPILEPSY

Many experimental models have been used in the study of human epilepsy, and there are several excellent reviews about their specific advantages.⁶⁻⁸ Among them, the kindling model of epilepsy is, in our opinion, the best model to study alternative therapeutic approaches to drug-resistant epilepsy. Originally described in rats, it has been reproduced in many mammals, including man, and even in amphibia.⁹ The characteristics of the kindling models and its usefulness to study new AED development are extensively reviewed elsewhere.¹⁰ Briefly, the model is achieved by periodic subconvulsive stimulation (usually electric) of a specific brain site.^{11,12} In the case of limbic epilepsy models, the target site is usually the amygdala, the hippocampus, or the entorhinal cortex.

The application of the first stimuli provokes a local afterdischarge with minimal or no electrical activity in the distant cortical electrical activity. After several stimulations, the animal shows characteristic electrical and behavioral features of focal seizures. Several stimuli later, these focal seizures are followed by secondarily generalized seizures. The pattern of progressive complex seizures was classified by Racine¹³ as follows:

Stage 1: Mouth and facial movements; Stage 2: Head nodding; Stage 3: Forelimb clonus; Stage 4: Rearing; and Stage 5: Rearing and falling.

Once the stage 5 is achieved, seizures will be provoked whenever this or another limbic is stimulated, even if the periodic stimulation stops. Apart from the increasing propagation of the afterdischarges, the kindled animals show a progressive diminution of the afterdischarge threshold, defined as the minimal current intensity needed to provoke an afterdischarge.¹³

The different variables that describe the kindling model (seizure stage, afterdischarge and seizure threshold, afterdischarge and seizure duration and latency) also differen-

tially describe the focal and the generalized components of the seizures.¹⁰ Thus, the kindling model is very useful to study the mechanism by which an anticonvulsant drug acts. Besides this, it has several characteristics that make it useful for the study of alternative methods to treat drug-resistant epilepsy. First, it mimics mesial temporal lobe epilepsy in humans, which comprises the most important group of drug-resistant epilepsies in humans. Indeed, although it responds to the same AEDs that control temporal lobe epilepsy in humans, some animals express resistance to these drugs, constituting a model of drug-resistant epilepsy under certain conditions.¹⁰ Also, it is convenient to study the local delivery of drugs, since the epileptogenic area is localized (the trigger zone corresponds to the place of electrode insertion), and the seizures are triggered by the experimenter. Further, as it is a chronic preparation, neurological toxicity can be tested using standard procedures, such as the rotarod or different neurological scales.¹⁴

Kindling can be achieved with different interstimuli intervals in the range between 30 min and 7 days.^{12,15} It is possible to develop rapid kindling by increasing the duration of stimulus trains at shorter intervals, but this kindled state is usually transitory.⁸

INTRACEREBROVENTRICULAR DRUG INFUSION

Our first experience with intracerebroventricular AED infusion was using the conventional drugs clinically useful for mesial temporal epilepsy injected acutely through the ICV route. These included phenytoin (PHT), carbamazepine (CBZ), and phenobarbital (PB). The most powerful anticonvulsant was PB, followed by CBZ and PHT. Both PB and CBZ caused significant reductions in seizure intensity to less than stage 3 with the doses tested, but PHT only reduced seizures for the first 15 to 30 min after application. However, the anticonvulsant efficacy dropped quickly within minutes after the injection. PB was also the most toxic drug, followed by CBZ and by PHT, and neurological toxicity also dropped quickly with time.¹⁶ These time profiles correlated with initially high drug concentrations at the ipsilateral hemispheres and lower at the contralateral hemisphere, brainstem, or cerebellum, which dropped quickly, together with the CSF concentrations, while plasmatic levels were low or undetectable. These profiles correlate well with the expected drug distribution associated with an acute ICV injection.⁴

Continuous intracerebroventricular infusion of 15% PB using an osmotic pump achieved good seizure control, with almost complete seizure suppression, at the cost of mild ataxia and sedation. PB drug concentrations were 381.4 $\mu\text{g/g}$ in the brain hemisphere corresponding to the infusion side, while they were 8.25 $\mu\text{g/g}$ in the

cerebellum. This drug distribution correlates properly with continuous drug infusion.

The good results obtained by drugs acting focally by a presumed gamma-aminobutyric acid (GABA)ergic mechanism led us to test intracerebroventricular injection of valproic acid in the kindling model, which had been recently formulated as a parenteral presentation.

In these experiments, acute injection of varying doses of ICV valproic acid suppressed seizures but resulted in remarkable ataxia and sedation, while continuous ICV infusion controlled generalized and even focal seizures without producing important side effects, high plasma levels, or hepatic drug concentrations.¹⁷ This may have important implications for a drug such as valproic acid, which may produce a lethal idiosyncratic hepatotoxicity.

The next candidate for ICV infusion was gabapentin, since it is a water soluble drug, a GABAergic drug with low neural toxicity and with a saturable transport mechanism through the BBB. Acute ICV injection of gabapentin proved to be more toxic and less effective than intraperitoneal injection. This may be explained by the existence of the CSF brain barrier and by the difficulty of diffusion by the thickness of the brain parenchyma in a short time.⁴ Continuous intraventricular gabapentin did not produce any anticonvulsant effects at low doses (50 mg during 7 days). Higher doses (total dose 100 mg during 7 days) had a greater impact on afterdischarge threshold and generalized seizure threshold, without any toxic effect. However, a dose escalation produced neurotoxicity without improvement in antiepileptic efficacy (unpublished data).

Drug distribution in the brain from the CSF is determined by a series of factors that include the diffusion of the drug from the CSF into the brain extracellular space, the diffusion of the drug into the blood capillaries, the uptake of the drug by neurons or glia, and drug metabolism in the brain. Pharmacokinetically, drugs that pass readily through the BBB achieve moderately high concentrations, but are rapidly cleared from the brain. For that reason, the characteristics that favor the passage of the BBB, such as lipid solubility, low polarity, or active carriers, may lead to an uneven distribution throughout the brain when given at the CSF.

In summary, intraventricular delivery may achieve anticonvulsant efficacy without systemic toxicity, but does not seem to improve neurologic toxicity. This may be due to the wide distribution of the drug in the CNS by means of intracerebroventricular infusion that includes the target areas for neurotoxicity.

INTRACEREBRAL INFUSION

Another different way to bypass the BBB is to use the brain as the route of administration of the drugs. This can be done with intracerebral catheters, either by passive

diffusion (as in microdialysis),¹⁸ or by active infusion (as in infusion enhanced delivery),¹⁹ or by the implantation of polymers, either degradable or nondegradable,²⁰ which slowly release the drug within the parenchyma. Catheter-driven infusion has been used both acutely and chronically in experimental and clinical settings.^{21,22} One of the studied alternatives has been the intracerebral infusion of inactivating substances to reversibly block a chosen structure.²³ Local anaesthetics, divalent cations, or sodium channel blockers have been tried. However, inhibitory transmitters or their agonists have been the ones obtaining a more controlled, more specific and less brain harmful inhibition. The direct infusion of the neuroactive drug in the precise brain area where it acts may be an appropriate therapeutic option.²⁴ This possibility has already been explored to treat epilepsy by several authors²⁵⁻²⁷ who found different effects over the initiation, spread, and behavioral features of seizures, depending on which was the area infused.

Election of the target area for brain infusion

One of the main questions in epilepsy surgery in general, and in drug infusion for epilepsy in particular, is the selection of the target point. The traditional idea of an epileptic focus as a discrete area of the brain where seizures originate has been challenged by the discovery of multiple networks whose synchronized activity underlie the epileptic phenomena, which are necessary to maintain epileptic activity.²⁸ Even though, experience from surgical resective procedures show us that removal or disconnection of certain brain areas can lead to seizure control. Thus, inactivation of part of the network system may be sufficient to deactivate at least part of the mechanisms responsible for seizure initiation or generalization.²⁹

Intracerebral infusion in the epileptic focus

Direct manipulation over the epileptogenic area is considered the most effective means to treat epilepsy.³⁰ On the other hand, the goal of a restorative treatment would be the re-establishment of the equilibrium between the inhibitory and excitatory neurotransmitter systems. Local restoration of GABA, or local bioavailability of GABA agonists would thus restore the local relative deficit of GABA, which is thought to underlie epilepsy.³¹ Indeed, GABA turnover is diminished in the amygdala kindling.³² Thus, GABA infusion can be considered as a restorative procedure.

Several studies have addressed this strategy. For example, local infusion of diazepam was effective to diminish seizures both in models of epilepsy by pilocarpine injection-induced seizures after local cortical cobalt application and in the bicuculline model.^{27,33,34}

The acute intra-amygdalar injection of muscimol, a GABA-A receptor agonist, or of antagonists of AMPA (α -amino-3-hidroxi-5-metil-4-isoxazol-propionate, one of

the glutamate receptors), also suppresses amygdala-kindled seizures.³⁵

The local infusion of other drugs or neurotransmitters, such as lidocaine or taurine, the glutamate antagonists, or the serotonin and 2-chloro-adenosine agonists, all of which act through non-GABAergic mechanism, has been effective in models of epilepsy, such as kindling.^{36–41} Also, epileptogenesis is impaired after infusion of inhibitory substances at target structures functionally related with the epileptogenic area, such as hippocampus²⁶ or the central pyriform cortex.^{42,43}

Although most of these experiments show anticonvulsant effects, local application of drugs have a limited effect span, sometimes of a few hours. Also, these studies have been developed in acute seizure-provoking models rather than in spontaneous seizures models.²⁹

Among the strategies sought to prolong the effect of the drug on the epileptic focus, self-degradable polymers liberating anticonvulsant drugs, such as phenytoin, have been tried.⁴⁴ Another option is to use a pump to continuously infuse the drug through a catheter. In this case, one of the candidate drugs for infusion can be GABA itself. Even though GABA itself may not be appropriate for its systemic administration, due to its low BBB pass,⁴⁵ this property may make it suitable for intracerebral continuous infusion.^{25,46}

In our group, we have investigated the effect of continuous intra-amygdalar infusion of GABA in the amygdala-kindling model of epilepsy in the rat. A cannula was attached to the bipolar electrode that was implanted in the left basolateral amygdala of adult male Wistar rats. Those animals were subjected to a standard kindling procedure. The continuous intra-amygdala infusion was achieved with osmotic mini-pumps filled with either GABA or mannitol that were connected to the cannulas. The infusion with these mini-pumps lasted 7 days and kindling experiments were performed before, during, and after. After-discharge and seizure thresholds, seizure severity and duration, and behavioral toxicity were measured. Both after-discharge and seizure thresholds were significantly increased both during and after GABA infusion with respect to pre-infusion controls, whereas the group receiving mannitol showed no significant differences. Seizure duration was not affected by GABA infusion. Seizures were either completely blocked or fully developed. We conclude that direct continuous GABA infusion within the epileptogenic focus raises the seizure thresholds without affecting the generalized components in the amygdala-kindling model of epilepsy.⁴⁷

Intracerebral infusion in pathways of propagation of seizures

Although infusion within the place where seizures originate would seem ideal, sometimes the location of this place can not be established or it can be extensive or

multifocal, or its inhibition can lead to adverse effects worse than the epileptic condition. In such cases, instead of the epileptogenic area, it may be better to inhibit the pathways that propagate epileptic activity to the rest of the brain or that activate brain areas that inhibit the epileptic activity.

Focal seizures of limbic origin propagate through anatomically well-defined pathways. The nucleus dorsomedianus thalami is one of the relays through which limbic structures output to the frontal cortex, and can play an important role in the spreading of these seizures.⁴⁸ Bilateral injection in both nuclei dorsomedianus thalami of glutamate antagonists, or GABA agonists, inhibits seizures provoked by systemic pilocarpine.⁴⁹ Also, injection of similar drugs in the ipsilateral nucleus dorsomedianus thalami inhibits generalization of limbic seizures provoked by acute application of bicuculin at the “area tempestas.”⁵⁰

Motor expression of seizures was suppressed in the amygdala-kindling model in rats by continuous infusion of GABA at the motor cortex.²⁵ In this experiment, however, the sudden withdrawal of GABA infusion led to seizures. Amygdala has been also shown to be a fundamental nucleus in seizure propagation. Infusion of GABA agonists in the amygdala dampens the expression of generalized seizures, with a minimal influence over the local postdischarges induced by kindling of the insular or entorhinal cortices.⁵¹ A different strategy is to stimulate areas which are known to be inhibitory for seizures. For example, stimulation of the GABAergic neurotransmission at the pars reticulata of the substantia nigra, considered to have an important role in seizure propagation,⁴⁸ has a strong anti-epileptic effect in several models of epilepsy in the rat.^{52,53} However, results contradicting this effect have also been published,⁵⁴ and the importance of this nucleus in seizure generalization is yet to be determined.⁵⁵

Other targets for drug infusion have been the nucleus reticularis pontis oralis involved in seizure expression in the maximal electroshock model in rats where N-methyl-D-aspartate antagonists have been infused,⁵⁶ or the periaqueductal gray for audiogenic seizures.⁵⁷ However, the transfer of these results to clinical practice is limited by the nature of the models used.

However, continuous infusion of drugs by external pumps may be subjected to long-term complications, such as mechanical failure, obstruction, infection, rejection, the need to refill its content, or lack of physiologic modulation of its activity.⁵⁸

BRAIN GRAFTING

Cell transplantation has appeared as a therapeutic opportunity in neurologic diseases,⁵⁹ including epilepsy. This can be viewed under two approaches: first, the implantation of cells releasing neurotransmitters or other

neuroactive drugs, which would then act as biological mini-pumps, and second, the repair of lost or degenerated tissue, whose lost cells are replaced.

Transplantation of GABA-releasing cells

In the first strategy, the implanted cells must not necessarily be neurons or neuronal precursors, but they must be able to release neuroactive substances.^{60,61} One of such substances is GABA, which can be released by genetically manipulated cells. It has been shown that such cells, genetically manipulated to produce and release GABA under a regulable promoter, are able to modulate electrically induced seizures after their transplantation into the substantia nigra,^{62,63} the pyriform cortex,⁶⁴ or the hippocampus.⁶⁵ Also, adenosine-producing cells have been shown to be effective.^{66,67}

However, this strategy is not free of problems, such as time-limited efficacy due to limited cell survival and down-regulation of neurotransmitter receptors at the host tissue, the host against graft reaction, or the potential uncontrolled proliferation of these cells, which may lead to the need to encapsulate them.

Transplantation of neural precursor cells

The second strategy is directed toward the lost cell replacement to restore function.⁶¹

Cell transplantation is able to hinder epileptogenesis in several models of epilepsy, including genetically epilepsy-prone rats, subcortically denervated hippocampi or kainic acid or pilocarpine injection models. In these cases, although the transplant has achieved effects on seizures, the mechanism of action is unclear.

Neurotoxin-induced lesions of neuromodulatory systems, noradrenergic, serotonergic, or cholinergic, have been used to facilitate the development of kindling. Transplantation of neural precursors delays the development of the kindling model, possibly by means of repairing those neuromodulatory systems.⁶⁸ Also, intravenous injection of human stem cells is able to lower the incidence of spontaneous recurrent seizures in a model of pilocarpine induced post-status epilepticus.⁶⁹ These cells are able to migrate to the hippocampus and differentiate into inhibitory GABAergic interneurons. However, the clinical relevance of these findings is unclear because those lesions are not characteristic features of human epilepsy.⁶⁸ The use of grafting as prevention of the development of kindling would limit its application to already instituted clinical epilepsy.²⁹

Transplantation of neural precursor cells may be also anticonvulsant in already epileptic animals. Grafts of hippocampal cells, obtained from 13 to 14 day rat fetuses into the hippocampi of kindled rats may suppress kindled seizures.⁷⁰ A greater efficacy was obtained when the implanted cells were embryonic stem cells.⁷¹ In this study, the generalization of seizures was progressively reverted after 4 weeks post-grafting, and the grafted cells

had acquired the phenotype of GABAergic neurons. On the contrary, neuroepithelial stem cells derived from a clonal mouse line in several models of epilepsy failed to show any improvement.⁷² Recent studies have succeeded in reducing frequency and intensity of recurrent spontaneous motor seizures in rats with chronic temporal lobe epilepsy, by means of intrahippocampal transplantation of hippocampal neural precursors from rat pups.⁷³

Another interesting source of GABAergic cells is the embryonic median ganglionic eminence, which is the source of adult GABAergic cortical neurons.⁷⁴ These cells are capable, when implanted into the postnatal brain, to migrate extensively and differentiate into adult inhibitory GABAergic neurons,⁷⁵ making functional synaptic connections.⁷⁶ This is in contrast with other neuronal precursors, which show only a limited migration once implanted in the adult brain.^{77,78}

The implant of fetal tissue rich in GABA has also been tried in structures related to the generalization of seizures, such as substantia nigra pars reticulata,^{25,79–81} although the effect had a short duration possibly due to loss of GABA release or downregulation of receptors with time.

All these experiments have led to the clinical application of porcine foetal GABA producing cells in three patients with focal epilepsy, with a good result in two of them.^{82,83} These studies have been stopped by the United States National Institutes of Health due to the concerns about the safety of patients, because of a possible transmission of porcine xenoviruses.⁸⁴

CONCLUSIONS

We may conclude that intracerebroventricular administration of anticonvulsants in animal models of epilepsy, specifically the kindling model, may lead to think of clinical applications in very specific contexts, such as the need to administer specific systemically toxic drugs. Intracerebral infusion may seem a more advantageous procedure, whether it is aimed at the epileptogenic area or to its generalization pathways. This approach may be limited by technical problems related to the chronically implantable pumps. An alternative to overcome this is to use grafts of neuroactive substance-releasing cells. This approach must be carefully balanced to the possible risks of implanting biologically active cells into the brain.

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