Chapter 16

The NIH Anticonvulsant Drug Development (ADD) Program: preclinical anticonvulsant screening project

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Summary

Summary reports are written for those compounds that have undergone multilevel evaluations. A report analyzes and interprets the generated data, and also compares the candidate compound's pharmacodynamic and pharmacokinetic profile to that of current clinically effective drugs. Annually, approximately ten promising compounds are considered for further development by the ADD Program. If a compound appears to have potential, the ADD Program collaborates with the pharmaceutical or academic sponsor in scheduling further preclinical and clinical evaluations.

Introduction

Many patients with epilepsy fail to experience adequate control of their seizures, despite the optimal use of available anti-epileptic drugs marketed in the United States, Other patients do so only at the expense of significant toxic side effects. Since 1975, the Epilepsy Branch of the National Institute of Neurological Disorders and Stroke, National Institutes of Health, through its Antiepileptic Drug Development (ADD) Program, has collaborated with the pharmaceutical industry in developing new therapeutic agents for the treatment of seizure disorders. In 1993, felbamate, the first new drug in nearly two decades, was approved for sale in the United States. Felbamate's development was a collaborative effort of both the pharmaceutical sponsor and ADD Program. Two other drugs, topiramate and remacemide were identified in the Branch's preclinical evaluation and are now in late clinical development. Topiramate is being developed worldwide and received market approval in the UK in 1995. Several other drugs, losigamone (Schwabe, Germany), D-23129 (Asta/AWD Germany), D2916 (Biocodex, France), CGP 33101 (Ciba-Geigy, Switzerland) and BW 534U (Wellcome-Glaxo, USA) are now in early clinical development.

The ADD Program boasts of both a preclinical and clinical trials component. The preclinical component consists of drug discovery and toxicology elements. These elements are carried out under contracts to the NIH. For the past two decades, the drug discovery project, known as 'The Anticonvudsant Screening Project' (ASP), has successfully identified numerous lead compounds for development. The ADD

Program's Toxicology Project provides additional support incentives to encourage industry's management to continue long term development of the very best compounds.

The initial preclinical evaluation of a compound's anticonvulsant potential is accomplished by screening new submissions through a series of *in* vivo and *in vitro* tests. Comparative decisions are made at each step in this multilevel testing process until the best compounds are selected. One of the unique aspects of the ASP is the diversity of chemical entities submitted for screening. Unlike an individual company that utilizes in-house drug libraries, the ASP receives compounds from a large and diverse group of suppliers from around the world. Over 300 worldwide suppliers have submitted their compounds to the ADD Program for evaluation. In recent years, ten to fifteen new suppliers have joined the ADD Program annually. At the present time, the ADD Program has 120 industrial and 196 academic suppliers providing compounds for evaluation. This broad spectrum of participants provides a large variety of new chemical entities. The diversity of structural submissions provides a unique opportunity to increase the chances of discovering compounds with new mechanisms of actions. All compounds of interest are compared and contrasted with known clinically effective drugs. It is believed that this process assures that the most novel drugs are proposed for clinical development.

The ASP was initiated at the University of Utah in the early 1974 under a NINDS contract. Over the past twenty years, an average of 865 new chemical entities have been screened annually for anticonvulsant activity. In early 1994, the ASP's multilevel testing procedures were revised from previous descriptions of the ADD Program's Screening Project (Kupferberg, 1989). The protocol revisions include both mechanistic and 'seizure type' models for evaluating a compound's anticonvulsant potential. Only a small number of compounds actually progress beyond the early identification phases and enter into the more advanced and complex evaluation procedures.

Compounds submitted by industrial suppliers enter into **the ADD Program** under a confidentiality agreement between the NIH and the supplier. This agreement assures the strict confidentiality of the compounds source, chemical structure and evaluation results. The Government can not release or publish any data without prior written approval from the sponsor. No therapeutic areas other than epilepsy are evaluated during the screening process. Compounds are given a unique identification code prior to its shipment to the ASP. The confidential data generated from the ASP's multilevel evaluation is filed with the Epilepsy Branch and then returned directly to the compound's sponsor. The sponsor has the final responsibility in the determination of their compound's development. In most cases, the sponsor seeks advise from the ADD Programs's staff in making their development decisions.

Anticonvulsant screening

A singular approach to identifying potentially useful drugs can not be used in a drug discovery program such as the ADD Program. For example, a **mechanistic** approach assumes a specific mechanism(s) for seizure initiation, propagation, and amelioration. Examples of this approach would be to identify compounds that work via inhibition of excitatory amino acid mediated excitation or enhancement of GABA-mediated inhibitory transmission. This approach also assumes that an appropriate model exists (usually an *in vitro* model). To some extent this approach limits the discovery of mechanistically novel substances. The advantage of this approach is that a large number of compounds can be evaluated in a short period of time, with limited amounts of material and without the use of large numbers of animals.

The **non-mechanistic** approach has advantages and disadvantages. Non-mechanistic seizure models have clearly defined endpoints. Examples of such endpoints are the inhibition of the tonic hindlimb extension phase, following electrically induced seizures or clonic seizures following administration of seizure producing doses of chemoconvulsants. These methods require limited technical expertise and they permit a direct comparison of the anticonvulsant profile of a new drug to that of the 'clinically effective therapeutic agents'. Unfortunately, this approach provides little pertinent information regarding an active compound's mechanism of action, which is highly desirable for making development decisions.

The ASP uses both electrical (MES) and chemical induced seizures (scPTZ) for its initial screening procedures. The animals are 'normal' and not genetically predisposed to seizures. While the neuronal sensitivity of normal mice to anticonvulsants may be different than in epileptic animals, these models afford certain advantages over genetic epileptic prone animal models for the following reasons: (1) They are suited to routine screening of a large number of potential anticonvulsants. (2) Normal animals are less expensive than are the inbred genetic animals. (3) Genetically epileptic prone rodents exhibit a greater number of false positive responses to non-anticonvulsant drugs. (4) The historical database for normal animals is extremely large allowing for large-scale comparisons.

The **'seizure-type** model' approach is limited because a limited number of 'Epilepsies' are associated with an animal model. The models that are available require a high degree of technical expertise, are costly and labor intensive. With such models, drugs for symptomatic epilepsy are not readily identified, whereas those for the generalized epilepsies are much more easily identified.

The Anticonvulsant Screening Project (ASP)

The ASP uses a combination mechanistic, non-mechanistic and 'seizure-type' approach to identify potential compounds for the treatment of seizures. The initial screening procedures are broad and non-mechanistic and serve to identify CNS and minimal neurotoxic activity of the compound. Once identified in the initial non-mechanistic screens, a compound's activity is then differentiated using **'syndrome-specific'** animal seizure models. Finally, advanced studies are used to identify proconvulsant potential of compounds, tolerance to the anticonvulsant effects, and possible molecular targets that can contribute to a compound's mechanism of action.

Primary evaluation

The ASP initially evaluates anticonvulsant activity for newly submitted compounds following intraperitoneal (i.p.) administration in mice and oral administration in rats. Two convulsant tests (MES and scPTZ) and a toxicity screen (rotorod in mice, positional sense and gait in rats) are employed for primary evaluation.

The Maximal Electroshock Seizure (MES) or Maximal Seizure Pattern Test

The MES is a model for generalized tonic-clonic seizures. It is highly reproducible with consistent endpoints. The behavioral and electrographic seizures generated in this model are consistent with the human disorder (Swinyard *et al.,* 1989). This model identifies those compounds which prevent seizure spread.

In the MES test, an electrical stimulus of 0.2 s in duration (50 mA in mice and 150 mA in rat at 60Hz) is delivered via corneal electrodes primed with an electrolyte solution containing an anesthetic agent. Mice are tested at 30 minutes and 4 hours following doses of 30, 100 and 300 mg/kg of test compound. Other doses can be used if previously known pharmacology merits deviation. Rats are tested at time intervals between 0.25 and 4 hours following a standard oral dose of 30 mg/kg. Abolition of the hindlimb tonic extensor component indicates the test compound's ability to inhibit MES-induced seizure spread (White *et al.,* 1995a; White *et* al., 1995b; Swinyard *et al.,* 1989).

The subcutaneous Pentylenetetrazol (Metrazol) Seizure Test (scPTZ)

This is a model that primarily identifies compounds that raise seizure threshold. The behavioral seizure produced is not typical of absence epilepsy but clonic in nature. Like other rodent models of absence seizures, PTZ induced seizures are potentiated by GABA agonist. With some minor exceptions, the pharmacological profile of the scPTZ seizure model is consistent with the human condition (Snead, 1992; Swinyard et al., 1989)

The scPTZ test utilizes a dose of pentylenetetrazol (85 mg/kg in Carworth Farms No. 1 mice and 70 mg/kg in Sprague-Dawley rats). This produces clonic seizures lasting for a period of at least five seconds in 97 per cent (CD_{97}) of animals tested. At the anticipated time of testing the convulsant is administered subcutaneously. The test compound is administered intraperitoneally in mice and orally in rats. Animals are observed over a 30 minute period. Absence of clonic spasms in the observed time period indicates a compound's ability to abolish the effect of pentylenetetrazol on seizure threshold (Swinyard et al., 1989). All clinically active anticonvulsants have been found to be protective in at least one of these two tests.

Minimal neurotoxicity

Toxicity induced by a compound is detected in mice using the standardized rotorod test described by Dunham & Miya (1957). Untreated control mice, when placed on a 6 r.p.m. rotation rod, can maintain their equilibrium for a prolonged period of time. Neurological impairment can be demonstrated by the inability of a mouse to maintain equilibrium for one minute in each of three successive trials.

Rats are examined for behavioral toxicity by the positional sense test and a gait and stance test. In the positional sense test, one hind leg is gently lowered over the edge of a table, whereupon the rat, experiencing neurological deficit, will fail to lift its leg quickly back to a normal position. In the gait and stance test, neurotoxicity is indicated by a circular or zigzag gait, ataxia, abnormal spread of the legs, abnormal posture, tremor hyperactivity, lack of exploratory behavior, somnolence, stupor or catalepsy.

Compounds that possess significant anticonvulsant activity in rats and mice and do not exhibit substantial neurotoxicity or death are considered for the ADD Program's multiphase evaluation to establish a compound's pharmacodynamic/pharmacokinetic profile.

During the past five years, over 4000 compounds have been evaluated by the ADD Program. Approximately 10 per cent of these compounds were active in the MES test following administration of doses as low as 30 mg/kg in mice. In the scPTZ test, 154 compounds were found to be active following i.p. administration of doses at or below 100 mg/kg in mice. In primary anticonvulsant screens for rats, 60

compounds produced complete protection against induced seizures in all animals in at least one of five time periods examined from 15 minutes to 4 hours at doses of 30 mg/kg.

Secondary evaluations

All quantitative in vivo anticonvulsant/toxicity evaluations of the most active compounds are conducted at a compound's time of peak pharmacodynamic activity (TPE). Groups of at least eight mice or rats receive various doses of the candidate compound until at least two points are established between the limits of 100 per cent protection or toxicity and 0 per cent protection or minimal toxicity. The 95 per cent confidence limits (95 per cent C.I.), slopes of the regression lines and standard errors of the slopes are calculated for each quantitative determination (Finney, 1971). Rats receive test compounds orally and mice intraperitoneally.

For the same five year period, 143 compounds had MES $ED_{50s} \leq$ 20 mg/kg and approximately 50 compounds had scPTZ $ED_{50s} \leq$ 20 mg/kg. As a number of active compounds were found in both species, discriminating questions and decision points were incorporated in an attempt to better identify lead compounds for further development and resource allotment.

Decision processes

Several considerations arise from the primary and secondary mouse screens. Does the compound produce death or possess proconvulsant potential? Is there significant separation between anticonvulsant activity and minimal neurotoxicity/death? If the compound is from a series, is it the most potent/least toxic? Is the compound's chemical structure unique? What is its duration of action? Finally, what kind of resources can be mobilized to assure the most expedient development.

From the early rat screens, the following questions can be posed. Does the compound produce complete protection (4/4) at 30 mg/kg at any time point? Does the compound produce neurotoxicity following oral administration? If not, should other routes of administration be used which may bypass possible absorption problems? If from a series, which compound is the most potent with the longest duration of activity? Is it structurally unique or similar to other anticonvulsants?

Tertiary/in-vivo evaluation

Lead compounds enter a more complex level of evaluation. The limited *in vivo* tests are used to elucidate at compound's inhibitory mechanism of action and therapeutic utility. The initial quantitative differential evaluations in mice include clonic seizures induced by the subcutaneous administration of bicuculline and picrotoxin (White, 1995b *et al., Swinyard et al.,* 1989).

The candidate compound's anticonvulsant activity in a genetically seizure prone animal model is then determined. Several types of genetically seizure prone animals are available to evaluate a candidate's ability to block the tonic phase of the reflex epilepsy. Specific strains of mice, rats, baboon, gerbils and chickens (Locher, 1984) have been used to determine a compound's anticonvulsant activity. The genetically susceptible Frings strain (Frings, 1952; Swinyard *et al.,* 1963) is used by the ADD Program for this purpose. This strain differs from the seizure susceptible DBA/2 strain in that it maintains its reflex epilepsy potential throughout its life. Individual mice are placed in a plexiglass cylinder and exposed to a sound stimulus of 100 decibels (11 Hz) for 20s. Anticonvulsant testing occurs at the compound's previously determined time of peak activity. The seizures experienced by these animals are characterized by wild

running, followed by loss of righting reflex with forelimb and hindlimb tonic extension. A compound's pharmacologic efficacy is demonstrated by the abolition of the tonic hindlimb extension.

The **seizure-type** models used in the ADD Program's evaluation of anticonvulsant activity are: (1) **Hippocampal kindling** model of focal seizures and (2) **Gamma (g)-hydroxybutyrate spike and wave** model of generalized absence seizures.

The hippocampal kindling model can be used to evaluate a compound's ability to affect both the **expression and acquisition** of focal seizures. The hippocampal kindling paradigm as described by Lothman and Williamson (Lothman, 1994) offers a distinct advantage over other kindling models. In particular, it is one of the only models wherein the temporal effects of a drug can be evaluated in a single animal. This procedure requires the surgical placement of bipolar electrodes in the ventral hippocampus of adult male Sprague-Dawley rats. Stage five behavioral seizures (Racine, 1972) are produced by using a stimulus consisting of a 50 Hz, 10 s train of 1 ms biphasic 200 μA pulses delivered every 30 min for 6 hours (12 stimuli per day) on alternating days for a total of 60 stimulations (five stimulus days). Prior to evaluating a candidate's anticonvulsant activity, a drug free control period consisting of supramaximal stimulations are recorded to verify the stability of a stage five generalized seizure.

A single dose of the candidate compound is then administered intraperitoneally (i.p.), 15 min following the last control stimulation. The anticonvulsant activity of the drug is assessed every 30 min for three to four hours starting 15 min after administering the test material. After each stimulation, individual Racine seizure scores and afterdischarge durations are recorded. Rats are used again in drug trials after four to five drug- and stimulus-free days.

In the kindling acquisition study, drugs are tested for their ability to prevent the development of the kindled state in electrode implanted rats. The candidate compound is administered during the kindling procedure. For these studies, drug is administered at a predetermined time prior to the electrical stimulus. The dosing interval and the dose of the drug are based on the compound's activity observed in the acute seizure expression studies. Results from drug-treated animals are compared to those of saline-treated rats. This treatment is repeated on stimulus days two, three, four, and five. After a stimulus-free interval of one week, the effect of prior drug treatment on kindling acquisition is assessed by challenging the animal with the kindling stimulus protocol. The standardized kindling protocol is then carried out with the behavioral seizure score and afterdischarge duration recorded for each rat during three 'retest days'. Saline treated rats are fully kindled at the first stimulation following the one week stimulus-free period. An active compound would be expected to lower behavioral scores and afterdischarge duration compared to saline control rats. The suppression or lengthening of the delay in the acquisition of the kindled response may indicate that the candidate compound can act to prevent the development of seizures. Such compounds could be termed 'antiepileptogenetic'.

g-hydroxybutyrate spike wave model

Selected phenytoin-like candidate substances are further evaluated in rats by the γ-hydroxybutyrate (GHB) spike-wave model of absence (Snead, 1992). The GHB model, like the i.v. PTZ test, is utilized to ascertain the proconvulsant potential of test substances which possess a phenytoin-like anticonvulsant profile in other models. Generally, eight rats are treated orally with the dose of candidate substance that provides 97 per cent protection against MES seizures; at the TPE, 150 mg/kg of GHB is administered i.p. The EEG of individual rats is monitored for a period of two hours and the severity of spike-wave seizures

evaluated by computer analysis of the EEG. This is also an extremely useful model for verifying whether candidate substances with an ethosuximide-like profile will possess activity in another model of absence (Snead, 1992).

Proconvulsant evaluation

A compound that prevents seizure spread (active in the MES test), can lower seizure threshold at the same time (White, 1995a). Thus, this compound would have proconvulsant activity while at the same time prevent generalized tonic seizures. Mexiletine, a cardiac antiarrhythmic drug, is a potent MES compound, yet it lowers seizure threshold at doses slightly above the MES ED_{50} in mice. The timed intravenous pentylenetetrazol seizure threshold test in mice identifies those MES compounds with proconvulsant potential (Orlof, 1949).

Mice are given the candidate substance intraperitoneally at the ED_{50} and TD_{50}. At the time of maximal MES activity, an intravenous infusion of 0.5 per cent heparinized solution of pentylenetetrazol (0. 34 ml/min) is started. The time to the appearance of the first myoclonic jerk and the subsequent sustained clonic seizure are the measured endpoints. Results are obtained for groups of ten treated and ten saline control mice and converted to the dose in mg/kg of PTZ necessary to produce the two endpoints. Proconvulsants lower the dose of PTZ required to produce the endpoint. Anticonvulsant drugs such as valproic acid, ethosuximide and phenobarbital increase the amounts of PTZ required to produce the above endpoints.

In-vitro mechanistic studies

Two mechanistically related methods are used to evaluate a compound's interactions with excitatory (glutamate) and inhibitory (GABA) receptor gated ion channels. The methodology uses whole cell patch-clamp electrophysiological measurement on single mouse cortical neurons. Whole cell recordings are obtained from the primary cultured neurons using borosilicate glass electrodes as described by Hamill *et al.* (1981). Results from these studies provide the first insight into the molecular mechanisms of action of candidate substances. The *in vitro* actions of these experiments are then correlated with the data from the animal seizure models.

Tolerance and metabolism studies

Finally, subchronic administration studies are initiated in order to provide preliminary information regarding a compound's ability to produce tolerance, hepatotoxicity and effects on drug P-450 metabolism isozymes. The in vivo tolerance studies consist of administering the candidate compound orally for five days to rats and then comparing the anticonvulsant activity to animals receiving the compound acutely, following four days of saline treatment. These studies employ four groups of eight rats per group. Two groups are treated for four days with vehicle alone. Two groups receive different doses of active material for four days. On the fifth day, each drug treatment group and one saline treated group receives the oral ED_{50} of the compound. The candidate compound's anticonvulsant activity for each group is determined. Activity in the chronically treated groups are compared to the acute treatment and saline control.

On day six, all groups are given hexobarbital (100 mg/kg) intraperitoneally and the sleeping times (time from loss to regaining of righting reflex) measured. The hexobarbital sleep time tests provides an assessment of hepatic drug metabolism. These data provide some indication of whether changes in observed anticonvulsant activity are due to either pharmacodynamic or pharmacokinetic interactions.

Treatment is continued for an additional day. The rats are then euthanized, blood collected and livers removed and weighed. The blood is allowed to clot and serum analyzed for alanine amino transferase (sALT) activity. Increased sALT may indicate possible liver damage. The excised livers are perfused in situ with saline, and homogenized in 0.25 M sucrose. Hepatic endoplasmic reticulum (i.e. microsomes) and cytosol are isolated following centrifugation. Microsomal cytochrome P-450 concentrations and both cytosol and several microsomal oxidative dealkylases, and transferases are then determined. Any metabolic enzyme induction is then correlated with any changes in anticonvulsant activity and hexobarbital sleep time.

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