Antiepileptic Drug Development Program[1]

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The modern era of antiepileptic drug therapy began with the use of phenobarbital in 1912. In the years thereafter, many new drugs were introduced, including other barbiturates, hydantoins, succinimides, and oxazolidinediones. Then, for various reasons, the marketing of new antiepileptic drugs was dramatically curtailed. To help reverse this trend, the Epilepsy Branch of the National Institute of Neurological and Communicative Disorders and Stroke sponsored clinical trials of drugs which had already been marketed abroad, resulting in the distribution of clonazepam, carbamazepine, and valproic acid in the U.S. These trials were followed by the establishment of the Antiepileptic Drug Development Program, which encompasses both the preclinical and clinical elements of drug development, including the Anticonvulsant Screening Project, the Toxicology Project, and support for controlled clinical trials.

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Approximately 2.5 million (1%) of all Americans have epilepsy; 200,000 have seizures more than once a month, making epilepsy second only to stroke as the leading neurological disorder. It often begins in childhood, with 75% of patients having their first seizure before the age of 18. Most patients are dependent on drugs for seizure control, but therapy is often inadequate. For some patients with intractable seizures, hope may lie in the development of more effective antiepileptic drugs; for others, a new drug may reduce the side effects they must often tolerate to gain seizure control with current treatment. The purpose of this paper is to describe the Antiepileptic Drug Develop-

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Table 1. Antiepileptic drugs marketed in the United States

Year introduced	International nonproprietary name	U. S. trade name	Company
1912	Phenobarbital	Luminal	Winthrop
1935	Mephobarbital	Mebaral	Winthrop
1938	Phenytoin	Dilantin	Parke–Davis
1946	Trimethadione	Tridione	Abbott
1947	Mephenytoin	Mesantoin	Sandoz
1949	Paramethadione	Paradione	Abbott
1950	Phethenylate*	Thiantoin	Lilly
1951	Phenacemide	Phenurone	Abbott
1952	Metharbital	Gemonil	Abbott
1952	Benzchlorpropamide†	Hibicon	Lederle
1953	Phensuximide	Milontin	Parke–Davis
1954	Primidone	Mysoline	Ayerst
1957	Methsuximide	Celontin	Parke–Davis
1957	Ethotoin	Peganone	Abbott
1960	Aminoglutethimide‡	Elipten	Ciba
1960	Ethosuximide	Zarontin	Parke–Davis
1968	Diazepam§	Valium	Roche
1974	Carbamazepine	Tegretol	Geigy
1975	Clonazepam	Clonopin	Roche
1978	Valproic acid	Depakene	Abbott
1981	Clorazepate dipotassium§	Tranxene	Abbott

* Withdrawn in 1952
† Withdrawn in 1955
‡ Withdrawn in 1966
§ Approved by the FDA as an adjunct

ment Program, the federal government's effort to develop more effective and less toxic drugs for epilepsy, and to review the highly successful era of the 1940s and 1950s when many major antiepileptic drugs were marketed, as well as the 12-year hiatus which took place between 1961 and 1973.

History of antiepileptic drug development

During the mid-1800s, a number of inorganic bromide salts were reported to produce good sedative effects and were accepted into medical practice. Potassium bromide, used by Locock to treat catamenial seizures,[1] largely replaced earlier drugs when it was found to reduce seizure frequency in many patients after other forms of therapy had failed. Although it was used regularly during the next 50 years, it was found to cause severe skin eruptions and psychosis, prompting a search for less toxic drugs.

The modern history of antiepileptic drugs marketed in the U.S. (*Table 1*) begins in 1912 with the introduction of phenobarbital, a synthetic sedative-hypnotic drug which was shown to reduce seizure frequency.[2] As it proved to be more effective and less toxic than potassium bromide, phenobarbital soon became the drug of choice. Since the barbituric acid molecule is easily modified, many analogues of phenobarbital were synthesized, of which approximately 50 were marketed in the first 35 years of this century. One of these analogues, mephobarbital, demonstrated good antiepileptic activity and was marketed in the U.S. in 1935.

In the absence of experimental models of seizures which could be used to test anticonvulsant activity, the discovery of the antiepileptic effect of bromide and phenobarbital was serendipitous. Later, with the development of seizure models, the search for new antiepileptic drugs was based on scientific screening programs.

One of the earliest models of epilepsy was developed in 1882, when seizures were elicited in dogs by direct faradic stimulation of the motor cortex and used to test chemicals for anticonvulsant activity.[3] Later, other seizure models involving convulsant chemicals were developed, including the naturally occurring picrotoxin, bicuculline, and strychnine and the synthetic compound, pentylenetetrazol. The use of systemic chemical convulsants as experimental models has been well described.[4]

The year 1937 marked the beginning of the experimental evaluation of promising anticonvulsant chemicals prior to clinical use. Using a seizure model based on a new electroshock technique for producing convulsions in animals,[5] Merritt and Putnam[6,7] screened a group of compounds supplied to them by Parke–Davis and discovered the anticonvulsant properties of phenytoin, then called diphenylhydantoin. Because phenytoin was well tolerated by laboratory animals, it was subjected to clinical trials in 1938 and marketed that same year. The absence of a sedative effect and the dramatic control of seizures observed when phenytoin was added to barbiturate therapy were the key factors in its rapid marketing. In addition, its entry into the market was not delayed by regulatory requirements, since at that time the introduction of new drugs was still regulated by the Federal Food and Drugs Act of 1906, which required only that drugs be accurately labeled without requiring proof of safety or efficacy.[8]

The reliability and quantitative capacity of Merritt's method demonstrated the feasibility of testing new chemicals for anticonvulsant activity.[6] Administration to humans, a more costly, time-consuming, and risky procedure, could confidently be reserved for the most effective experi-

mental compounds that emerged from such testing programs. In addition, the process through which phenytoin came onto the market demonstrated that academic investigators could work successfully with the pharmaceutical industry, encouraging a relationship that flourished for the next 20 years.

Several pharmaceutical firms began molecular modification projects dealing with phenytoin and its analogues, and numerous hydantoins were synthesized and tested during this period. In addition, investigators from the pharmaceutical industry as well as academic researchers began to explore new and improved methods of provoking seizures.

In 1944, Richards and Everett[9] reported that trimethadione, a potent analgesic compound that was to become the first anti-absence drug, prevented pentylenetetrazol-induced threshold seizures in rodents. They also showed that these seizures were prevented by phenobarbital, but not by phenytoin. Goodman et al[10] confirmed these results and showed that phenytoin and phenobarbital modified the pattern of maximal electroshock seizures while trimethadione did not. These findings demonstrated the varying anticonvulsant actions of these drugs and the qualitative difference between threshold and maximal seizures.

Between 1945 and 1950, several investigators conducted tests with a variety of seizure models, but failed to find one in which all drugs were active. However, these tests uncovered profiles of anticonvulsant activity which, with few exceptions, correlated well with clinical efficacy and specificity.[11] In 1951, Chen et al[12] investigated the anticonvulsant activity of approximately 65 phenylsuccinimides and found that among the most potent antipentylenetetrazol compounds were phensuximide and methsuximide, both of which were later approved for treatment of absence seizures (1953 and 1957, respectively). A third succinimide, ethosuximide, was introduced for the same purpose in 1960.

During the same period (1938–1960), two analogues of phenytoin (mephenytoin and ethotoin), two of phenobarbital (metharbital and primidone), and one of trimethadione (paramethadione) were marketed in the U.S. Each had a spectrum of activity comparable to that of its parent drug and was marketed for similar use.

Three other drugs, phethenylate, benzchlorpropamide, and aminoglutethimide, were also introduced during this period; however, both

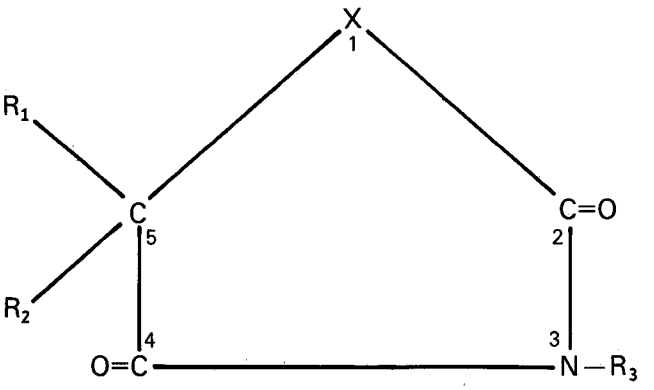

Fig. 1. R_1, R_2, and R_3 indicate different side-chain members. *X* refers to components of different drug groupings: hydantoinates (*-NH-*), barbiturates (*-CO-NH-*), oxazolidinediones (*-O-*), succinimides (*-CH_2-*), and acetylureas (*-NH_2-*).

phethenylate and benzchlorpropamide were withdrawn by 1960, as the former was associated with a high incidence of hepatic necrosis and the latter demonstrated toxic effects with long-term use in experimental animals. Aminoglutethimide was withdrawn in 1966 after it was linked to a high incidence of goiter.

Interestingly, all antiepileptic drugs developed from 1912 to 1960 were based on a simple heterocyclic ring structure (*Fig. 1*). During this period, genuinely novel structures were ignored in the development of antiepileptic drugs; instead, attention centered on the hydantoins, barbiturates, oxazolidinediones, succinimides, and acetylureas.

After 1938, marketing of all drugs in the United States was regulated by the Federal Food, Drug, and Cosmetic Act,[13] which required proof of safety in addition to the 1906 labeling provisions. Definitive questions about efficacy usually were not resolved until after a new drug was marketed.

Decline in antiepileptic drug development

The highly productive era of antiepileptic drug development in the 1940s and 1950s was followed by a dormant period lasting for 12 years, from 1961 to 1973, during which the only new drug of interest was diazepam, an adjunctive drug used mostly in status epilepticus (*Table 1*). There were many reasons for this. For one thing, some clinicians believed that improvements in therapy depended mainly on better use of existing drugs, and this belief helped strengthen the impression that there was not a substantial need for new drugs, even though many patients with common types of seizures and most of those with

rare types failed to respond to available drugs and many suffered side effects. Furthermore, the attention of the pharmaceutical industry shifted to other areas of central nervous system (CNS) therapy following the remarkable financial success of tranquilizers and sedative-hypnotic drugs. In addition, because of the relatively large number of effective drugs already available, many representatives of the pharmaceutical industry questioned whether a new drug could capture a large enough market to justify the cost of development. This cost, already on the increase, rose still more steeply with the addition of the Drug Amendments Act of 1962[14] to the 1938 regulation. Known as the Kefauver–Harris amendment, this legislation required that efficacy be established as a prerequisite for marketing approval in the U.S. and restricted the conditions under which drugs could be tested. It had a serious impact on drug development, not only for epilepsy but also for any other disease affecting a relatively limited population, wherein the market was correspondingly small and the return on corporate investment doubtful.

Problems with clinical testing also contributed to the reluctance of pharmaceutical firms to develop new drugs. For example, proof of the efficacy of new antiepileptic drugs was seriously hampered by the almost total lack of patient populations whose seizure types and frequencies were well defined. Many patients withdrew from controlled clinical studies following either dramatic improvement or increased seizures. In addition, the common use of multiple drugs complicated the design of controlled clinical trials that could establish the efficacy of a new drug used alone. Moreover, many clinicians did not believe in the need for controlled trials, and the resulting lack of scientific data was a major impediment to the development of new antiepileptic drugs according to the newer, more rigorous standards of the 1960s. A 1967 survey of pharmaceutical firms[15] revealed that most had no new antiepileptic drugs under development due to prohibitive cost, while several drugs had not gained the approval of the U.S. Food and Drug Administration (FDA) because of inadequate proof of efficacy. Furthermore, an informal survey of academic medicinal chemists revealed that those synthesizing potential anticonvulsant agents had no access to appropriate pharmacologic testing.

Renewed interest in antiepileptic drugs

Beginning in 1968, the Epilepsy Branch of the National Institute of Neurological and Communicative Disorders and Stroke, in collaboration with other investigators, attempted to reverse the decline in antiepileptic drug development by conducting controlled clinical trials of seven drugs (albutoin, carbamazepine, clonazepam, clorazepate dipotassium, mexiletine, sulthiame, and valproic acid) that needed proof of efficacy before they could be marketed in this country. Support of these trials not only decreased the cost of development for the pharmaceutical industry, but also provided an opportunity for the Epilepsy Branch to develop a methodology and standards for the conduct of such trials. The resulting data eventually supported new drug applications (NDA) for carbamazepine, clonazepam, and valproic acid, which were marketed in this country as primary antiepileptic drugs in 1974, 1975, and 1978, respectively, and for clorazepate dipotassium, which was approved in 1981 as an adjunctive drug for treatment of epilepsy. Trials of albutoin, mexiletine, and sulthiame failed to support their efficacy in the populations studied, and these drugs have not been marketed in the U.S.

In addition to clinical trials of available drugs, the need for involvement in the development process at the preclinical stage soon became apparent, and a federally sponsored antiepileptic drug development program was formally established with the introduction of the Anticonvulsant Screening Project in 1975.

Antiepileptic Drug Development Program

Several other government-sponsored drug development programs set the precedent for federal assistance in antiepileptic drug development. While these programs vary, they are concerned primarily with drugs that, for various reasons, would not be developed independently by drug companies. The two fundamental elements of such programs are (*a*) preclinical screening, often seeking potential drugs in an effort to entice drug companies to become interested in them, and (*b*) controlled clinical trials of new drugs.

The Antiepileptic Drug Development (ADD) Program, sponsored by the Epilepsy Branch, encompasses both the preclinical and clinical elements of drug development (*Fig. 2*). The preclinical segment comprises the Anticonvulsant Screening Project (ASP) and the Toxicology Project, and the clinical element is represented by sponsorship of controlled clinical trials. During the preclinical process, the ADD program receives compounds from both academic medicinal chemists and the pharmaceutical industry for screening, which is divided into seven phases (see

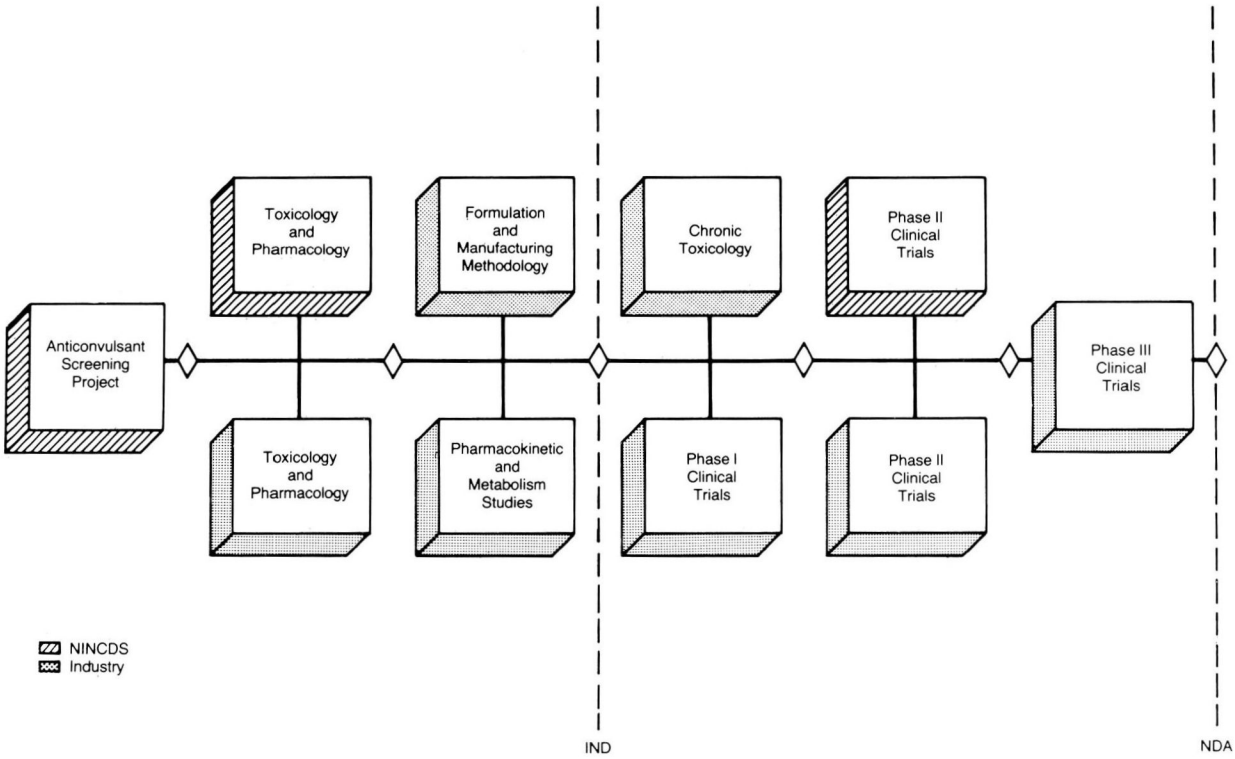

Fig. 2. Antiepileptic drug development process

above). When a compound shows exceptional anticonvulsant activity in the later phases, toxicology studies are recommended to the sponsor. In general, no drug can be administered to humans until one study has been conducted in rodents and another in other mammals (usually dogs), with the ADD program supporting one study and the sponsor supporting the other. The clinical segment of the program begins after the sponsor is granted an investigational exemption for a new drug (IND). Studies in healthy volunteers (phase I) are supported by the sponsor, or infrequently by the ADD program. Initial controlled clinical trials in epileptic patients (phase II) are supported by the ADD program. Finally, broader clinical trials (phase III) are conducted by the sponsor. Successful completion of clinical evaluation is generally followed by marketing of the drug.

To avoid confusion, the seven phases of the ASP are designated by Arabic numerals (phases 1–7), and the three phases of clinical evaluation are designated by Roman numerals (phases I–III).

Anticonvulsant Screening Project

Academic chemists and representatives of the pharmaceutical industry submit compounds to the ASP for evaluation of anticonvulsant activity, description of neurotoxic effects, and delineation of possible mechanisms of action (*Fig. 3*). The testing is done at no cost to them, and in addition, they retain the patent rights. As of October 1983, more than 8,100 compounds have been tested in a standardized, consistent manner, resulting in a data base by which the structure–activity relationships of other anticonvulsants can be predicted. The seven phases of the ASP are described in *Table 2*. The most commonly used antiepileptic drugs marketed in the United States were tested in phases 2–6, and the results are shown in *Table 3*.

Methodology: The clinical usefulness of the currently available antiepileptic drugs is indicated by their ability experimentally to prevent the spread of seizures and/or increase the minimal seizure threshold. Those applicable to generalized tonic–clonic seizures and partial seizures, such as phenytoin, prevent the spread of seizures and may or may not increase the minimal seizure threshold, while those used against absence seizures, such as ethosuximide, elevate the threshold and have little or no ability to prevent spread. Thus initial screening involves (*a*) the maximal electroshock seizure test to detect agents that prevent spread of seizures, and (*b*) the seizure

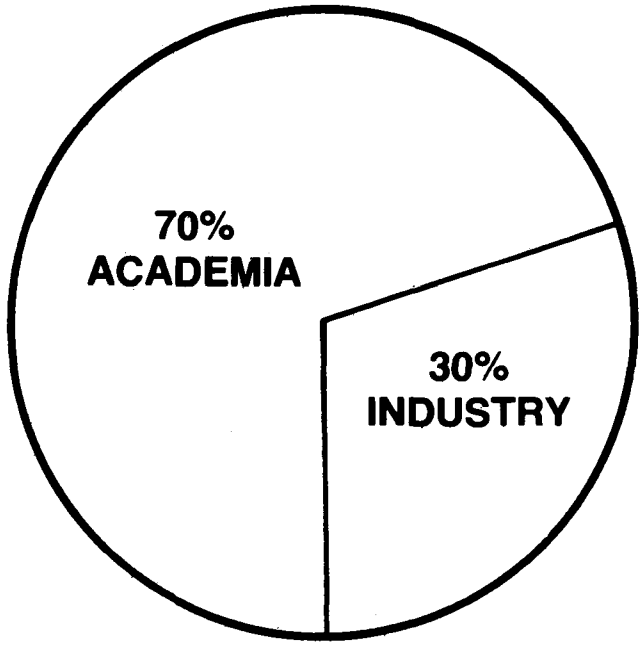

Fig. 3. Sources of the ADD program compounds

threshold test with subcutaneous pentylenetetrazol (Metrazol) to detect agents that elevate the minimal seizure threshold. If a compound shows significant anticonvulsant activity and demonstrates minimal neurotoxicity on the rotorod ataxia test, further screening is performed. All compounds tested are either dissolved in 0.9% sodium chloride or suspended in a mixture of 30% polyethylene glycol 400 and 70% water. Except for a specific interaction between certain drugs and polyethylene glycol in the Metrazol test, which results in increased activity of the test compound, the solvents introduce no significant bias. The compounds are administered intraperitoneally (i.p.) or orally (p.o.) to Carworth Farms #1 mice (in a volume of 0.01 ml/g of body weight) or Sprague–Dawley rats (in a volume of 0.004 ml/g of body weight). Times of peak effect and peak neurologic deficit are determined before the anticonvulsant tests are administered.

In the *maximal electroshock seizure test (MES)*, corneal electrodes primed with a drop of electrolyte solution (0.9% sodium chloride) are applied to the eyes and an electrical stimulus (50 mA in mice, 150 mA in rats; 60 Hz) is delivered for 0.2 second at the time of peak effect of the test compound. The animals are restrained by hand and released at the moment of stimulation in order to permit observation of the entire seizure. Abolition of the hind-leg tonic-extensor component (hind-leg tonic extension does not exceed a 90° angle to the plane of the body) indicates that the compound can prevent MES-induced seizure spread.

In the *subcutaneous pentylenetetrazol seizure threshold test* (*scMet*), the convulsant dose (CD_{97}) of pentylenetetrazol (85 mg/kg in mice, 70 mg/kg in rats) is injected at the time of peak effect of the test compound. The animals are isolated and observed for 30 minutes to see whether seizures occur. Absence of clonic spasms persisting for at least five seconds indicates that the compound can elevate the pentylenetetrazol-induced seizure threshold.

In the *subcutaneous bicuculline seizure threshold test* (*scBic*), the CD_{97} of bicuculline (2.70 mg/kg) is injected into mice at the time of peak effect of the test compound. They are then isolated and checked for seizures for 30 minutes. Absence of seizures indicates that the compound can elevate the bicuculline-induced seizure threshold.

In the *subcutaneous picrotoxin seizure threshold test* (*scPic*), the CD_{97} of picrotoxin (3.15 mg/kg) is injected into mice at the time of peak effect of the test compound, after which the mice are isolated and any seizures noted for 45 minutes. Absence of seizures indicates that the compound can elevate the picrotoxin-induced seizure threshold.

In the *subcutaneous strychnine seizure pattern test* (*scStr*), the CD_{97} of strychnine (1.20 mg/kg) is injected into mice at the time of peak effect of the test compound. The mice are placed in isolation cages and observed for 30 minutes for the hind-leg tonic-extensor component of a seizure; abolition of this component indicates that the compound can prevent strychnine-induced spread of seizures.

Acute anticonvulsant drug-induced toxicity in laboratory animals is usually characterized by some type of neurologic abnormality. In mice, these abnormalities are easily detected by the *rotorod ataxia test*, which is somewhat less useful in rats. When a normal mouse is placed on a knurled rod rotating at 6 rpm, it can maintain its equilibrium for a long time. The neurologic deficit is indicated by inability to maintain equilibrium for one minute in each of three trials. Rats are examined by the *positional sense test* and *gait and stance test.* In the positional sense test, one hind leg is gently lowered over the edge of a table, whereupon the animal will quickly lift it back to a normal position. Inability to do so rapidly indicates a neurologic deficit. In the gait and stance test, a neurologic deficit is indicated

Table 2. Test phases in the Anticonvulsant Screening Project

Phase 1:	Anticonvulsant identification to determine the level of activity [active (≤100 mg/kg) to inactive (>300 mg/kg)] (mice, i.p.)
	1. Maximal electroshock (MES) test—seizure spread
	2. Subcutaneous pentylenetetrazol (scMet) test—seizure threshold
	3. Rotorod ataxia test—neurotoxicity
Phase 2:	Anticonvulsant quantification to determine the level of activity at the ED_{50}, TD_{50}, and protective index (TD_{50}/ED_{50}) (mice, i.p.)
	1. Maximal electroshock (MES) test—seizure spread
	2. Subcutaneous pentylenetetrazol (scMet) test—seizure threshold
	3. Rotorod ataxia test—neurotoxicity
Phase 3:	Toxicity profile to assess general behavior and selected pharmacologic response at toxic doses (mice, i.p.)
	1. Median lethal dose (LD_{50})
	2. Median hypnotic dose (HD_{50})
Phase 4:	Anticonvulsant quantification to measure activity by the usual clinical route of administration and indicate the absorption and metabolic characteristics of the compound (mice, p.o.)
	1. Maximal electroshock (MES) test—seizure spread
	2. Subcutaneous pentylenetetrazol (scMet) test—seizure threshold
	3. Rotorod ataxia test—neurotoxicity
Phase 5:	Antiepileptic drug differentiation and comparison with known effective drugs to help determine the mechanism of action (mice, i.p.)
	1. Pentylenetetrazol seizure threshold test
	2. Picrotoxin seizure threshold test
	3. Bicuculline seizure threshold test
	4. Strychnine seizure threshold test
	5. Special in vitro receptor binding studies on selected candidate compounds
Phase 6:	Anticonvulsant quantification to measure activity in another species at the ED_{50}, TD_{50}, and protective index (TD_{50}/ED_{50}) (rats, p.o.)
	1. Maximal electroshock (MES) test—seizure spread
	2. Subcutaneous pentylenetetrazol (scMet) test—seizure threshold
	3. Positional sense test—neurotoxicity
	4. Gait and stance test—neurotoxicity
Phase 7:	Estimation of minimal lethal dose (LD_3) and effect of prolonged administration on anticonvulsant activity (rats, p.o.)
	1. Estimated LD_3 in male and female rats following administration once a day for 5 days
	2. Administration for 5 days—tolerance
	3. Hexobarbital sleep time test—tolerance
	4. Microsomal enzyme studies in vitro—tolerance

Abbreviations: i.p. = intraperitoneally, ED = effective dose, TD = toxic dose, p.o. = orally.

by a circular or zigzag gait, ataxia, abnormal spread of the legs, abnormal body posture, tremor, hyperactivity, lack of exploratory behavior, somnolence, stupor, or catalepsy.

Testing protocol: The potency and protective index of active compounds are estimated, and inactive or toxic compounds are eliminated from further testing in *phase 1* (anticonvulsant identification in mice, i.p.). Testing is carried out in 16 mice at doses of 30, 100, 300, and 600 mg/kg (4 mice apiece) 30 minutes and four hours after administering the compound. Based on the results of this test, compounds are divided into four groups: (*a*) those with no anticonvulsant activity at doses up to 300 mg/kg, which are not tested further; (*b*) those showing activity at 100 mg/kg, which are tested further; (*c*) those showing activity at 300 mg/kg, which may or may not be tested further depending on the novelty of the structure; and (*d*) those demonstrating activity and/or toxicity at 30 mg/kg, which are usually retested and may or may not be evaluated further. About 15% of all compounds are advanced to phase 2.

Phase 2 (anticonvulsant quantification in mice, i.p.) measures the anticonvulsant activity and neurotoxicity estimated in phase 1. The median effective dose (ED_{50}) is determined using the MES and scMet tests and the median toxic dose (TD_{50}) using the rotorod ataxia test. When a compound appears to exhibit anticonvulsant activity in one test, it is tested in the other model up to doses that produce a neurologic deficit. Whenever possible, compounds failing to produce a minimal neurologic deficit are tested to doses ten times their lowest anticonvulsant ED_{50}. The median effective dose is determined at the time of peak effect in the MES test except when preliminary testing indicates that scMet activity occurs at a different time; TD_{50} is determined at the time of peak neurologic deficit. The most promising compounds emerging from phase 2 become candidates for advanced testing.

Phase 3 (toxicity profile in mice, i.p.) reveals

Table 3. Results of the Anticonvulsant Screening Project

Compound		Mice, i.p. (phase 2)			Mice, p.o. (phase 4)			Rats, p.o. (phase 6)			Mice, i.p. (phase 5)				Mice, i.p. (phase 3)	
		MES (ED_{50})*	ScMet (ED_{50})	Tox (TD_{50})	MES (ED_{50})	ScMet (ED_{50})	Tox (TD_{50})	MES (ED_{50})	ScMet (ED_{50})	Tox (TD_{50})	ScBic (ED_{50})	ScPic (ED_{50})	ScStr (ED_{50})	ScMet (ED_{50})	Righting reflex (HD_{50})	Lethality test (LD_{50})
Phenytoin		9.5	N.E.†	65.5	9.0	N.E.	86.7	29.8	N.E.	>3000	N.E.	N.E.	Max prot	N.E.	178.3	229.6
	Slope	13.7		15.2	6.3		13.0	2.8					50% at 55–110 mg/kg		14.0	15.9
Mephenytoin		60.5	30.5	153.8	65.9	36.3	353.9	18.1	21.7	85.7	124.1	101.0	Max prot	30.5	406.0	568.0
	Slope	8.0	4.8	9.2	28.8	3.6	6.6	3.6	8.4	13.5	2.0	7.4	50% at 70–150 mg/kg	4.8	17.0	9.8
Phenobarbital		21.8	13.2	69.0	20.1	12.6	96.8	9.1	11.6	61.1	37.7	27.5	95.3	13.2	135.5	264.7
	Slope	15.0	5.9	24.7	5.2	3.8	8.5	4.1	4.1	3.0	4.1	4.8	18.5	5.9	8.4	16.0
Primidone		11.4	58.6	679.7	26.9	28.2	484.6	6.2	15.4	233.9	148.1	341.7	N.E.	58.6	720.7	734.9
	Slope	3.4	3.3	25.2	3.4	7.3	10.7	4.2	2.8	6.3	3.6	2.6		3.3	7.6	8.0
Trimethadione		627.5	300.5	819.1	1012.5	233.7	1326.9	1012.5	233.7	1326.9	532.1	408.1	Max prot	300.5	1689.2	2511.8
	Slope	11.4	14.3	8.2	15.8	10.1	16.2	15.8	10.1	16.2	16.5	7.4	37% at 500–1,500 mg/kg	14.3	10.2	15.2
Ethosuximide		>1000	130.4	440.8	>2000	192.2	879.2	>1200	54.0	1012.3	459.0	242.7	Max prot	130.4	850.6	1752.2
	Slope		10.1	18.4		7.4	30.5		9.1	15.3	3.2	26.4	62% at 250–1,000 mg/kg	10.1	16.4	14.8
Methsuximide		76.3	68.3	187.6	163.4	130.2	511.2	45.7	25.3	59.8	127.4	210.8	Max prot	68.3	376.8	789.6
	Slope	10.5	11.6	9.6	15.3	5.8	11.0	4.0	4.4	5.1	5.1	16.0	62.5% at 250 mg/kg	11.6	17.5	12.6
Diazepam‡		19.1	0.17	7.3	51.3	0.31	24.3	214.8	1.3	14.8	1.2	1.2	13.0	0.17	206.7	882.0
	Slope	6.4	6.5	1.5	6.7	2.9	6.5	2.8	1.8	8.4	1.5	9.1	1.0	6.5	8.2	6.5
Clonazepam		86.6	0.02	0.18	78.4	0.06	3.4	186.0	0.06	71.6	0.009	0.04	N.E.	0.02	>6,000	>6,000
	Slope	2.3	13.9	14.4	2.3	3.0	2.5	1.2	1.2	3.7	1.4	3.5		13.9		
Carbamazepine		8.8	N.E.	71.6	15.4	48.1	217.2	8.5	N.E.	813.1	N.E.	37.2	78.8	N.E.	172.2	628.7
	Slope	3.6		4.8	9.1	5.5	3.5	4.5		6.1		3.9	2.9		5.9	10.1
Valproic acid		271.7	148.6	425.8	664.8	388.3	1264.4	489.5	179.6	280.3	360.0	387.2	293.0	148.6	885.5	1104.6
	Slope	12.8	11.9	20.8	18.2	8.1	4.8	2.9	8.6	4.6	7.5	8.4	11.8	11.9	12.5	11.4

* ED_{50}, TD_{50}, HD_{50} and LD_{50} in mg/kg
† N.E. = not effective
‡ Adjunct only
Abbreviations: i.p. = intraperitoneally, p.o. = orally, MES = maximal electroshock seizure, ScMet = subcutaneous pentylenetetrazol (Metrazol), Tox = toxicity, ScBic = subcutaneous bicuculline, ScPic = subcutaneous picrotoxin, ScStr = subcutaneous strychnine, ED = effective dose, TD = toxic dose, HD = hypnotic dose, LD = lethal dose, Max prot = maximum protection.

the dose–time relationships with regard to overt toxic manifestations and determines the median hypnotic dose (HD_{50}) and the 24-hour median lethal dose (LD_{50}). Toxicity is determined by administering the TD_{50}, two times the TD_{50}, and four times the TD_{50}. Mice are observed for onset, intensity, and nature of overt toxicity at 10, 20, and 30 minutes and 1, 2, 4, 6, 8, and 24 hours after administration of the test compound, which in turn, can help clarify its effects on the central and autonomic nervous systems. The aforementioned neurotoxicity tests are also performed, with abnormal results on at least two of them indicating overt neurologic toxicity.

Phase 4 (anticonvulsant quantification in mice, p.o.) provides the same kind of information as phase 2, except that the test compound is given orally instead of intraperitoneally to see whether this makes any difference in the activity of the drug. The time of peak effect indicates how rapidly it is absorbed, while the ED_{50} and TD_{50} disclose how adequately it is absorbed, which is important because antiepileptic drugs are usually given by mouth. Consequently, test compounds that reach this stage and still exhibit a satisfactory anticonvulsant activity, margin of safety, and adequate absorption usually proceed to phase 5, particularly if they also have a novel chemical structure.

Phase 5 (antiepileptic drug differentiation in mice, i.p.) delineates antiepileptic potential in vivo and in vitro. The in vivo portion tests the compound in seizures induced by pentylenetetrazol, bicuculline, picrotoxin, and strychnine. Because each of these convulsants acts via a somewhat different neurotransmitter system, the resulting ED_{50}s may reflect the activity profile of the test compound, which can be compared with those of clinically effective drugs. In the in vitro portion, receptor binding of the compound is correlated with its anticonvulsant activity. This involves evaluation of its ability to displace radiolabeled flunitrazepam and gamma-aminobutyric acid from membranes that have been isolated as a P_2 fraction from whole-mouse-brain homogenates using standardized ultracentrifuge techniques. The estimated displacing potency of the compound is given as K_i (affinity constant of the inhibitor) and IC_{50} (inhibitor concentration that displaces 50% of the radiolabeled ligand from the membranes).

Phase 6 (anticonvulsant quantification in rats, p.o.) was added to the protocol to verify anticonvulsant activity and neurotoxicity in another rodent species. The ED_{50}s in the MES and scMet tests and the TD_{50} are determined after oral administration of the compound, and the positional sense test and gait and stance test are used to determine neurotoxicity. These studies help to determine whether the accumulated experimental data are promising enough to warrant moving the candidate compound into toxicity studies.

Phase 7 (estimation of minimal lethal dose and effect of prolonged administration on anticonvulsant activity in rats, p.o.) provides the dosage information which is essential for subsequent toxicity studies in the Toxicology Project as well as an indication of the development of tolerance, and hence is limited to those compounds showing the greatest antiepileptic potential. Both male and female albino rats are used in order to correlate drug response with sex. First the minimal lethal dose (LD_3) is estimated following oral administration once a day for five days. Any pharmacologic or toxic manifestations, including death, are recorded one and four hours after administration and before the next day's dose. Development of tolerance is first measured in 24 rats divided into three groups of eight. Group 1 receives the ED_{50} once a day for five days, with anticonvulsant activity at the time of peak effect being determined on the fifth day using either the MES or scMET test. Group 2 receives saline or suspension media for four days, followed by the ED_{50} on day five, with anticonvulsant activity being determined by MES or scMet at the time of peak effect. Group 3 is given saline or suspension media for five days and tested on the fifth day. A greater number of seizures in group 1 than in group 2 indicates the development of tolerance. Another method of determining tolerance is the hexobarbital sleep time test, which measures total duration (in minutes) of loss of the righting reflex in male rats following intraperitoneal administration of a hypnotic dose of hexobarbital on day six. Development of tolerance is indicated by a shorter sleep time in group 1 and may reflect induction of microsomal hepatic enzymes. Following completion of the sleep time test, the same rats are each treated with the original regimens for two more days before being killed. The liver is removed and weighed and the endoplasmic reticulum oxidative enzyme (hepatic microsome) isolated. Changes in liver weight, total liver protein, cytochrome P-450, enzyme activity of *p*-nitroanisole demethylase, and cytochrome C reductase are measured, and these in

vitro findings are used to confirm the results of the in vivo studies.

Toxicology Project

If the results from all phases of the ASP are favorable, the ADD program (with the assistance of a priority evaluation by the Epilepsy Advisory Committee) schedules compounds for further testing in the Toxicology Project, which provides the toxicity evaluation required by the FDA before humans can be exposed to the drug. Selection of compounds for toxicity studies is based on four criteria: (*a*) adequate absorption after oral administration to mice and rats; (*b*) adequate protective indices after oral and intraperitoneal administration to mice and rats; (*c*) a compound with a novel chemical structure; and (*d*) absence of tolerance to the anticonvulsant effects. The greatest emphasis is given to potent compounds having a novel structure, i.e., compounds whose activity is equal to or better than that of known drugs and belonging to a different chemical family. Toxicity studies consist of a 91-day evaluation of the compound after oral administration to rats and beagles. The compound is administered in doses exceeding those anticipated for clinical use. Urine, blood, and tissue samples from more than 25 organs are examined for abnormal changes, and any cardiovascular or autonomic abnormalities are noted.

Since one of the goals of the ADD program is cost sharing between the government and the pharmaceutical industry, the ADD program assumes only part of the cost of the toxicology studies. Before the studies begin, both the ADD program and the sponsor commit themselves to the time, money, and resources needed for this stage of drug development. The sponsor must synthesize kilogram quantities of the candidate compound and assure its purity and stability before toxicity testing as well as perform some pharmacologic studies on the renal, gastrointestinal, and cardiovascular systems. If a compound from an academic supplier is selected for toxicology studies, that supplier usually seeks a sponsor from the pharmaceutical industry.

Controlled clinical trials

Once a compound has progressed through all of the aforementioned tests, it is potentially ready to be used in humans. Nevertheless, because of important species-related differences in drug metabolism and action, the ultimate value of any new drug obviously must be proved clinically. FDA regulations require that clinical investigations be conducted in three phases: determination of safety in humans, usually healthy volunteers (phase I), tests to see whether the drug treats or prevents the disease for which it is intended, as well as estimation of clinical safety and efficacy (phase II), and evaluation of long-term efficacy and safety in extensive clinical trials (phase III). Generally speaking, two adequate and well-controlled phase II trials by independent investigators (or a multiclinic study in which data from at least three investigators can be evaluated independently) are considered minimal in establishing the efficacy of a new drug. Although not required, it is usually implied that at least one trial must be performed in the U.S. if the drug is to be approved for marketing in this country.

Phase I clinical trials: Phase I studies provide data on the safety, pharmacologic effects, pharmacokinetics, and side effects of a given drug. These studies are usually performed by the pharmaceutical company rather than the ADD program, since they do not require expertise in clinical epilepsy research. However, performance of these studies is entirely within the scope of the ADD program, especially if it would accelerate clinical testing of a highly promising compound or if the pharmaceutical company is unable to perform such studies on a timely basis, as for example if it is a foreign company lacking a firm understanding of the need for extensive phase I studies.

Usually, healthy ("normal") adult volunteers are involved in phase I testing. Since abnormality need not be considered, subjects are more readily available and interpretation of the findings is easier; however, the results may have limited application to those patients for whom the drug is being developed. Healthy volunteers may differ from patients in their ability to tolerate side effects from large doses of antiepileptic drugs; informed consent must be obtained, and close observation and expert supervision are mandatory. The investigators must be experienced in clinical pharmacology and medicine and be willing to perform the necessary tedious, frequent, and thorough examinations. Phase I studies usually consist of both single- and multiple-dose tests following a random-assignment, single-blind, or double-blind design, since antiepileptic drugs may produce CNS effects that are difficult to evaluate objectively.

Phase II clinical trials: Phase II studies are used to evaluate the safety and efficacy of a drug

Table 4. International classification of epileptic seizures[16] *

- I. Partial seizures (beginning locally)
  - A. Simple partial seizures (consciousness not impaired)
    - 1. With motor symptoms
    - 2. With somatosensory or special sensory symptoms
    - 3. With autonomic symptoms
    - 4. With psychic symptoms
  - B. Complex partial seizures (with impairment of consciousness)
    - 1. Beginning as simple partial seizures and progressing to impairment of consciousness
      - a. With no other features
      - b. With features as in simple partial seizures
      - c. With automatisms
    - 2. With impairment of consciousness at onset
      - a. With no other features
      - b. With features as in simple partial seizures
      - c. With automatisms
  - C. Partial seizures secondarily generalized
- II. Generalized seizures (bilaterally symmetrical, without local onset)
  - A. 1. Absence seizures
    - 2. Atypical absence seizures
  - B. Myoclonic seizures
  - C. Clonic seizures
  - D. Tonic seizures
  - E. Tonic–clonic seizures
  - F. Atonic seizures
- III. Unclassified epileptic seizures (data inadequate or incomplete)

* Approved by the International League Against Epilepsy in September 1981.

in epileptic patients and to determine the therapeutic dose range and its variability in individual patients. The ADD program has concentrated on support of phase II trials, at first sponsoring the trials which led to the marketing of four drugs from 1974 to 1981 and, more recently, evaluating promising compounds emerging from the screening and toxicology projects.

Design and execution of controlled clinical trials of antiepileptic drugs are not trivial tasks. The subtleties of seizures and clinical observations require close interaction beween clinical neurologists experienced in controlled clinical testing and biostatisticians experienced in the design of clinical trials and with knowledge of neurologic disease. The patient population must be carefully defined, and any practical limitations imposed on the study design by the problems of patient availability must be considered. A small patient sample is typical. Highly efficient statistical designs and accompanying analyses are imperative in testing new forms of antiepileptic therapy if the trials are to yield maximal clinically useful information.

Accurate classification of the type of seizure is crucial to success. Because several terms are used to describe seizures, the type of seizure must be clearly defined in order to make the trial comprehensible to others. Usually this is based on the description given by the patient or an observer, but this is often inadequate. For drug trials, the type of seizure should be diagnosed by video monitoring whenever possible and the seizures categorized according to the International Classification of Epileptic Seizures[16] (*Table 4*). In addition, because some patients experience more seizures than others, antiepileptic drug testing requires maximal statistical efficiency with minimal danger to the patient. The classic two-period crossover design (*Fig. 4*) has become a standard means of alleviating this problem.

Clinical trials are further complicated by the fact that many potential participants have several types of seizures, and a drug which is effective for one type may have no effect on another. Other antiepileptic drugs may be required when multiple types of seizures coexist, but most are enzyme-inducers and may affect the metabolism of the test drug. While it would be desirable to market a drug for all types of seizures it is effective against, the cost of mounting controlled studies for several types of seizures is considerable. In practice, an NDA is usually sought for a specific type of seizure and additional indications are requested later. The parameters used to measure drug efficacy must be sensitive and meaningful indicators of active treatment effect; sophisticated statistical analyses of poorly defined clinical

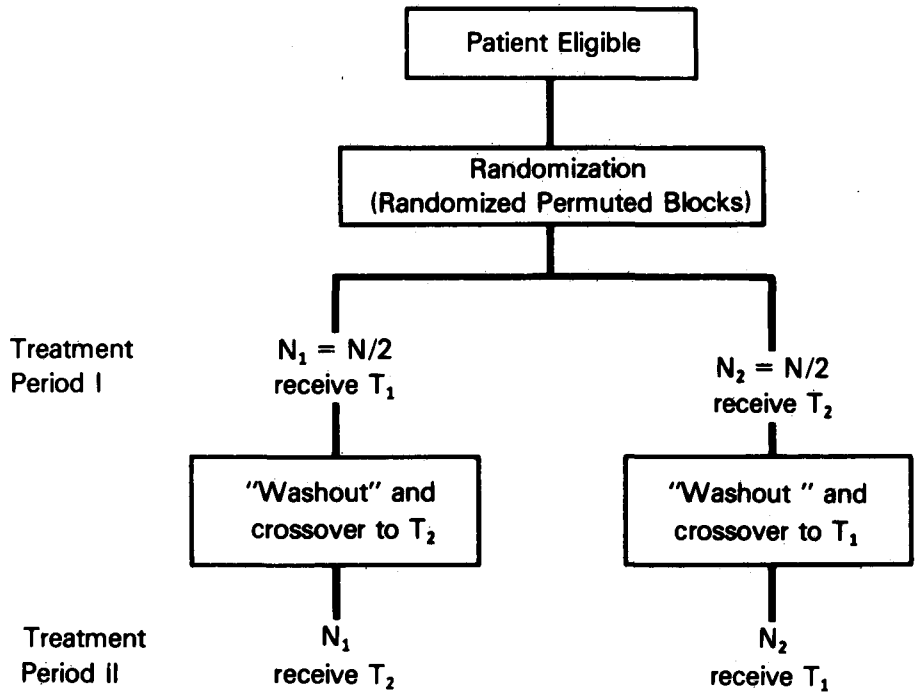

Fig. 4. Classic two-period crossover design of clinical drug trials. T_1 = standard treatment; T_2 = new treatment.

parameters could lead to studies which, in themselves, are appropriately analyzable but have very little inferential credence.

Finally, moral and ethical considerations limit testing of new drugs for some types of seizures to individuals who are continuing to have them despite optimal therapy with existing drugs, or who are experiencing severe adverse reactions to these drugs. Comparison of a test drug to a placebo is usually the best way of revealing drug effects; however, use of a placebo alone is only rarely permissible because of medical and ethical objections to not treating seizures. Thus in clinical trials of antiepileptic drugs, there is an increased emphasis on the need for clinically relevant end points and an effective statistical design. The ADD program has played a major role in advancing clinical trial methodology to help solve these problems. Controlled trials and double-blind studies are now the general practice,[17] and guidelines for antiepileptic drug testing have been developed by the Epilepsy Branch and adopted by the International League Against Epilepsy as well as the FDA.[18]

Phase III clinical trials: Phase III studies evaluate the long-term efficacy of new drugs, usually in patients treated successfully during the phase II trials. In an open protocol, about 150 of these patients are generally allowed to continue on the new drug for at least a year. Phase III studies are conducted by the pharmaceutical firm without the ADD program's support.

Ongoing clinical trials: Currently, the ADD program is supporting multicenter phase II clinical trials of four potential new antiepileptic drugs. Studies of progabide (a GABA agonist) at the University of Virginia and University of Minnesota, begun in 1981, are nearing completion. In 1982, a trial of an imidazole was started at the University of Washington and University of California at Los Angeles, and a trial of a carboxamide was started at the University of Michigan and University of Utah. Following pharmacokinetic studies at the University of Washington, a dicarbamate will be tried late in 1983 at the University of Virginia and the University of Minnesota. Preliminary studies of other promising compounds are also underway with the evaluation of a benzisoxazole at the University of Washington and a pyridine derivative at the Clinical Center of the National Institutes of Health.

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