Effects of Polytherapy Compared With Monotherapy in Antiepileptic Drugs: An Animal Study

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ABSTRACT

Although monotherapy in epilepsy treatment is frequently advocated, this is not based on studies with equal drug loads. This study was performed to investigate the experimental background of polytherapy with standardized drug loads. Dose-dependent effects on grip strength, accelerated performance, and spontaneous behavior of rats was used to study the effect of combining valproate and ethosuximide. The potency of the drugs (combination) was obtained by fitting the sigmoid E\textsubscript{max} equation to the data. Drug interaction was assessed using the isobologram method and quantified by comparing equivalent drug loads with their 95% confidence intervals. We found that the effects of valproate and ethosuximide combine in a simple additive way in the grip strength experiment as well as in the accelerated experiment. In the behavioral studies, however, a higher drug load of the combination was needed to obtain the same amount of sedation, signifying infra-additivity. Infra-additivity of sedative effects is an important finding because this is by far the most frequent side effect mentioned in human studies. However, assessment of the therapeutic effect of the combination will have to be completed before a preference for mono- or polytherapy, based on the balance of adverse effects and efficacy, can be expressed.

In the past, polytherapy was frequently used in antiepileptic drug therapy. This changed around 1980 when Reynolds and Shorvon started to advocate the use of monotherapy instead of polytherapy. In a series of open studies, they found little evidence indicating advantages of polytherapy, and, indeed, found that polytherapy was associated with more toxicity (Reynolds and Shorvon, 1981). A number of other investigators confirmed these findings. Fischbacher (1982), for example, studied well-being during use of antiepileptic drugs and found an improvement after reducing the number of anti-epileptic drugs without reducing the effect of therapy.

In these studies, however, standardized drug loads of the different therapy regimens were not equal, which is an important flaw when comparing two groups (Deckers et al., 1997). Lammers et al. (1995) standardized drug loads to calculate the total drug load of drug combinations. Their study showed that polytherapy does not imply more side effects at equal drug loads. However, randomized controlled clinical trials comparing antiepileptic drug combinations have not been carried out yet.

Perucca and Goldsmith and Bittencourt (Goldsmith and de Bittencourt, 1995; Perucca, 1995) emphasize the potential merits of rational polytherapy because there are still patients that do not sufficiently benefit from monotherapy. The term rational is used to emphasize that pharmacological principles are used as a basis to combine drugs. Hence, drugs with different types of working mechanism are combined to potentiate the therapeutic strength without potentiating toxicity or to reduce the side effects without reducing efficacy.

Polytherapy with antiepileptic drugs has been studied in animal models. Bourgeois (1990) studied the possible advantages of many antiepileptic drug combinations in mice. Anticonvulsant effect was studied by observing suppression of clonic seizures elicited by maximal electroshock or pentyleneetrazole (depending on the drug or drug pair to be studied) and neurotoxicity by the Rotarod test. Results were presented as a therapeutic index, i.e., a ratio of the concentration with toxic action in 50% of the animals and the concentration with therapeutic action in 50% of the animals. Four pairs of drugs were shown to have advantages when used in combination: phenytoin and phenobarbital, primidone and phenobarbital, valproate and carbamazepine, and valproate and ethosuximide (Bourgeois, 1988). Because of the low therapeutic index for phenobarbital, only the latter two were...
truly advantageous. In both cases, the anticonvulsant effect was purely additive, but due to an infra-additive neurotoxicity, the combination had a better efficacy versus toxicity ratio than the single drugs. However, neurotoxicity was only evaluated by use of the Rotarod, measuring motor coordination and praxis, and, for example, sedative effects were not assessed.

In the present study we focus on the combination of valproate and ethosuximide and on a more extensive evaluation of neurotoxic effects; grip strength meter, accelerating Rotarod, and video observation were used to assess neurotoxicity. Dose-effect curves of valproate and ethosuximide in mono- and polytherapy were determined to assess drug interaction with respect to strength, ataxia, and sedation. Also, a novel approach for the statistical analysis of drug interactions is presented.

Materials and Methods

Animals. Male adult Wistar rats weighing between 224 and 320 g were used for this experiment. They were housed in identical plastic cages and had free access to food and water except during the motor experiments. They were kept on a reversed light-dark cycle (dark between 0900 and 2100 h).

Drugs. Valproate (Albic Inc., Maassluis, the Netherlands) and ethosuximide (Sigma Chemical Co., the Netherlands), dissolved in 0.9% sodium chloride, were administered i.p. alternating left and right to prevent adhesions.

Experiment. The animals were divided in four groups of eight rats, one group receiving valproate, one group receiving ethosuximide, one group receiving the drug combination, and one saline control group. Every rat received six dosages, including a zero dosage, of the drug it was randomly assigned to, with an interval of 7 days. This interval was chosen on basis of the half-life of elimination of valproate (Loscher, 1978) and ethosuximide (Bachmann et al., 1988), being 4.6 and 22 h, respectively. The sequence of the six different doses was assigned to an individual rat according to an adapted Latin square. This design was chosen to correct for follow-up effects. All injections were blinded for the investigator. The dose of valproate ranged from 0 to 560 mg/kg and of ethosuximide from 0 to 360 mg/kg. For the drug combination, a fixed weight ratio of two-thirds valproate with one-third ethosuximide was given, and the doses ranged from 0 to 360 mg/kg valproate with 180 mg/kg ethosuximide. The doses and the ratio of valproate and ethosuximide were based on the amount of drug causing 50% of maximum effect (TD50) found in a pilot experiment. After weighing and injection, the rats were tested. The side effects were quantified with three tests.

First, the grip strength of the forepaws was determined (Kulig and Lammers, 1991). The grip strength apparatus consists of a push-pull strain gauge attached to a T-bar. To measure the grip strength, the animals were tested for 25 min by video camera between 100 and 125 min after injection. The animals were in observation cages of 30 × 30 × 50 cm and a minimum of light was used to keep them in an active state. The videotapes were observed with help of “The Observer” computer program (Noldus Information Technology Inc., Wageningen, the Netherlands). The behavior was categorized into four classes, namely: 1) active behavior, which included all movements except automatic behavior, such as locomotion, sniffing, and rearing; 2) passive behavior, defined as the absence of any movement; 3) grooming, and 4) automatic behavior, such as eating and drinking.

Data Analysis. The data of all three tests were analyzed by nonlinear regression analysis using the program GraphPad Prism 2.0 (GraphPad Software, Inc., San Diego, CA).

The data were fitted to the sigmoid E\textsubscript{max} model:

\[ E_{\text{drug}} = E_{\text{max}} \left(1 + \frac{E_{\text{max}} - E_{\text{min}}}{1 + \frac{\text{dose}}{TD50_{\text{min}}}} \right) \]  

E\text{drug} is the measured effect of the drug at a certain dose. E\text{drug} \text{starts at E_{min} and goes to E_{max} with a sigmoid shape. The TD50 and Hill factor (Hill) were calculated for the three drugs (valproate, ethosuximide and for the used drug combination according to its total weight).}

Note that TD50 in our experiment indicates the dose that gives half-maximal effect and not, as in the experiments of Bourgeois (1990), a dose that gives an end point effect in 50% of the animals.

Next, the theoretical additive curve was generated for the drug combination using the sigmoid E\text{max} model for a mix of two compounds according to eq. 2:

\[ E_{\text{combination}} = E_{\text{min}} + \frac{E_{\text{max}} - E_{\text{min}}}{1 + \frac{(A)^{\text{dose}} + (1-A)^{\text{dose}}}{TD50_{\text{valproate}} + TD50_{\text{ethosuxide}}} \text{compound}} \]  

with

\[ \text{Hill}_{\text{combination}} = \frac{(A)}{TD50_{\text{valproate}} + (1-A)} \times \frac{(1-A)}{TD50_{\text{ethosuxide}}} \]  

E\text{combination} is the calculated additive effect of the combination of drug at a certain dose. E\text{combination} \text{starts at E_{min} and goes to E_{max} with a sigmoid shape. A is the fraction of valproate in the combination. The Hill combination is the weighted mean of the Hills values of the single compounds. Next, the sigmoid E\text{max} model of eq. 1 was fitted to the generated additive data yielding an expected additive TD50 and an expected additive Hill. Confidence intervals (CIs) of the expected additive parameters are calculated from the CIs of the measured single compound curves using the equation:

Expected CI

\[ = [(A \times \% CI_{\text{valproate}} + (1-A) \times \% CI_{\text{ethosuxide}}) \times \text{expected TD50}] \]

The experimental parameter estimates of the drug combination were compared with the theoretical additive parameter estimates using the 95% CIs.

The curves of valproate and of ethosuximide were normalized using their TD50 values. The curve of the experimental combination was normalized using the TD50 of the theoretical additive combination.

The TD50 parameter estimates obtained by the sigmoid E\text{max} model were plotted in an isobologram to visualize the type of inter-
action (Tallarida, 1992). In an isobologram, the dose of one drug (valproate) is represented on the abscissa; the dose of the other drug (ethosuximide) is represented on the ordinate. Each plotted point in the graph represents a pair of doses of the two drugs that reach the TD50 when added in combination. The line that connects the two plotted points of the pure single drugs is the isobolographic line. If experimentally determined data points lie on this line, then the drug effects are additive (no interaction). If the points lie below this line, then there is supra-additivity (synergy), and if they lie above this line, then there is infra-additivity (antagonism).

**Fig. 1.** Change in grip strength. While fitting the curves to the data, the tops of the curves were fixed at 100% and the bottoms were fixed at 0%. For all four figures, dose response graphs for the change of side effects in rats following i.m. injection of valproate (open circles), ethosuximide (open squares) or of the combination of both (closed circles), with the normalized drug doses (dose/TD50) on the abscissa and the quantified data of the behavior on the ordinate. TD50 values (Tables 1–4) were obtained by fitting the sigmoid $E_{\text{max}}$ model (eq. 1) to the data. The solid lines show the theoretical additive dose response curves as derived from eq. 2, and the broken lines show the experimentally measured dose response curves. Parameter estimates are given in Tables 1 through 4. The insets show the TD50 values plotted in an isobologram with the TD50 of valproate on the abscissa and the TD50 of ethosuximide on the ordinate. The straight line that connects the two plotted TD50 values of the pure single drugs is the isobolographic line, with the dotted lines marking their 95% confidence intervals (CIs). Indicated with an * are the theoretical additive TD50 values which would be obtained with the used ration. The closed circles indicate the experimental TD50 values with their 95% CIs. If experimentally determined data points lie on the isobolographic line, then the drug effects are additive (no interaction). If the points lie below this line, then there is supra-additivity, and if they lie above this line, then there is infra-additivity. If the 95% CI of the experimentally determined combination do not overlap with the intervals of the isobolographic line, then the interaction is assumed to be statistically significant. Hill is a measure for the steepness of the curve.

**Results**

**Baseline Measurements.** To obtain baseline values and to correct for time effects, the grip strength and the accelerod performance were measured each test day before injection. A group × time analysis of variance was performed on these data. A group difference was present ($F$ (3144) = 6.51, $P < .001$ for the grip strength, and $F$ (3144) = 7.11, $P < .001$ for the accelerod). A day difference was present for the grip strength only ($F$ (5144) = 2.39, $P < .05$). No group × time interaction was present, indicating that the changes over time are the same in all groups.

For the grip strength, the group means varied from 970 g (S.E.M., 30 g) in the drug combination group to 1160 g (S.E.M., 30 g) in the saline control group. For the accelerod performance the group means varied from 110 s (S.E.M., 11 s) in the ethosuximide group to 171 s (S.E.M., 6 s) in the valproate group. An increase of grip strength was found over the test days, from 980 g (S.E.M., 30 g) on test day 1 to 1130 g (S.E.M., 50 g) on test day 6. Because of this time effect, we used the percentage postinjection performance of preinjection performance as the measure for drug effects for the grip strength and the accelerod performance. In this way, every rat functioned as its own control.

**Grip Strength.** The overall mean preinjection grip strength was 1060 g (S.E.M., 20 g). Both compounds as well as the combination negatively influenced grip strength performance in a dose-dependent fashion (Fig. 1). Equation 1,

**TABLE 1**

<table>
<thead>
<tr>
<th>Side effects quantified by grip strength</th>
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<tbody>
<tr>
<td>Valproate</td>
</tr>
<tr>
<td>TD50 (mg/kg)</td>
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<tr>
<td>95% CI</td>
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<tr>
<td>Normalized TD50</td>
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<tr>
<td>95% CI</td>
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<tr>
<td>Hill</td>
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<tr>
<td>95% CI</td>
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</table>

Parameter estimates of fitting the sigmoid $E_{\text{max}}$ curve to the data. The TD50 is the dose at which 50% toxic effect is achieved. In the drug combination, the total drug weight is indicated and is composed of two-thirds valproate and one-third ethosuximide. All weight values are normalized using the TD50 values. The experimental combination value uses the theoretical additive TD50 as norm. A normalized TD50 of the experimentally determined combination below 1 points to supra-additivity, whereas more than 1 indicates infraadditivity. If the 95% CIs of the normalized TD50 of the experimental combination do not overlap with the intervals of the theoretical additive ones, then the interaction is assumed to be statistically significant. Hill is a measure for the steepness of the curve.

**Fig. 2.** Change in accelerated performance. While fitting the curves to the data, the tops of the curves were fixed at 100% and the bottoms were fixed at 0%. For additional details, see legend to Fig. 1.
the sigmoid $E_{\text{max}}$ model, was fitted to the data, yielding the TD$_{50}$ and the Hill. With these parameter values of the single drugs, the theoretical additive curve for the drug combination was calculated using eq. 2. The grip strength data (Table 1) show that the experimental dose needed to get 50% toxicity in the combination experiment is lower than the theoretical additive dose, suggesting supra-additivity in toxicity. However, the 95% CIs of the experimental TD$_{50}$ and the theoretical TD$_{50}$ overlap to a great extent; therefore, this finding is not statistically significant (Table 1).

**Accelerod Performance.** The pre-experiment training required that every animal could stay on the accelerod for 60 s. The overall mean preinjection accelerod performance was 140 s (S.E.M., 5 s). Both compounds as well as the combination negatively influenced accelerod performance in a dose-dependent way (Fig. 2). Equation 1, the sigmoid $E_{\text{max}}$ model, was fitted to the data, yielding the TD$_{50}$ and the Hill. With these parameter values of the single drugs, the theoretical additive curve for the drug combination was calculated using eq. 2.

The accelerod data (Table 2) show that the experimental dose needed to get 50% toxicity in the combination experiment is higher than the theoretical additive dose, suggesting infra-additivity in toxicity. However, the 95% CIs of the experimental TD$_{50}$ and the theoretical TD$_{50}$ do overlap; therefore, this finding is not statistically significant (Table 2).

**Observation of the Behavior.** Both compounds caused, in a dose-dependent way, the animals to be less active (Fig. 3) and more passive (Fig. 4) than the saline control animals. Grooming and automatic behavior was not influenced in a dose-dependent way and no further inference was performed on these data. Equation 1, the sigmoid $E_{\text{max}}$ model, was fitted to the data, yielding the TD$_{50}$ and the Hill. With these parameter values of the single drugs, the theoretical additive curve for the drug combination was calculated using eq. 2. The experimental TC$_{50}$ values of the drug combination are higher than the theoretical additive ones for both the passive behavior and the active behavior (Tables 3 and 4). In both cases, the 95% CIs of the experimentally measured TD$_{50}$ and the theoretical TD$_{50}$ do not overlap. Thus, a statistically significant infra-additivity was found for these toxic effects of combination therapy.

**Discussion**

Various methods have been used for the analysis of drug interactions. In an extensive review, Berenbaum (1989) lists the most commonly used approaches, such as the isobologram method, the summation of effects-method, the multiplication of surviving fractions method, the method of calculating the effect of a zero-interactive combination from the law of mass action, and the often-used “no method approach” (i.e., authors claiming to have demonstrated supra-additivity or synergy without specifying their methods). Berenbaum convincingly shows the isobologram method, which was created by Fraser (1872) and further developed by Loewe (1953), to be the most valid method. Berenbaum (1989) claims that the greatest advantage of this method compared with others is that interactions can be analyzed ‘irrespective of their mechanism of action or the nature of their dose-response relationships’.
In this experiment, we used the isobologram method to evaluate the interaction of valproate and ethosuximide on adverse effects. Loss of strength, as measured by the grip strength meter, and loss of coordination, as measured by the accelerod, combined in an additive way. However, accelerated performance only became significantly affected at the highest dose level. Observation of behavior shows significantly more active and less passive behavior in polytherapy compared with monotherapy, indicating infra-additivity. These two measurements are not totally complementary because grooming and automatic behavior were also measured. The fact that the behavioral studies show significant infra-additivity in toxicity is an important finding when translated to humans, because sedation is the most frequently reported side effect of antiepileptic drug therapy (Collaborative Group for the Study of Epilepsy, 1986). Furthermore, our experiments may reflect clinical experience that adverse effects become apparent earlier in spontaneous behavior than in elicited behavior, as exemplified by the accelerated results.

However, how this infra-additivity for sedation may be explained in terms of mechanism of action is uncertain. Ethosuximide reduces the low-threshold (T-type) calcium current of thalamic neurons at clinically relevant concentrations, whereas valproate has no effect on this current in these neurons (Coulter, 1989). In another study, however, Kelly et al. (1990) did show valproate to modestly reduce the low-threshold (T-type) calcium current, albeit in primary afferent neurons. Other mechanisms of action of valproate are also still a matter of debate. Some studies point to an increase in γ-aminobutyric acid (GABA) in the brain or a postsynaptic potentiation of the GABA response. Others point to a direct effect on neurons by interference with the sodium channel or activation of calcium-dependent potassium conductance (Farriello et al., 1995). The infra-additivity of sedation in our experiment does at least suggest that the two drugs cause sedation by different mechanisms.

There is no consensus on whether rational drug combinations should work on the same neurotransmitter system or not (Leach, 1997). One may hypothesize that when two drugs work on the same system, an even greater effect may be achieved, and thus, lower dosages would be needed, implying less toxicity. For example, Klitgaard et al. (1993) reported that two drugs working differently on the GABA system had a supra-additive antiepileptic effect when combined, whereas combining a glutamate receptor antagonist and a GABAergic drug had no such effect. In the aforementioned experiments of Bourgeois, supra-additivity for antiepileptic effect was only accomplished in two cases (primidone + phenobarbital, phenobarbital + phenylethyl malonamide). Three of the four cases in which infra-additivity for neurotoxic effects were achieved were combinations of drugs which reportedly have different mechanisms of action. Although the combination of valproate and ethosuximide was only additive for the antiepileptic effect in Bourgeois’ animal model (1988), Rowan et al. (1993) described five patients with refractory absence seizures who became seizure-free only after receiving this combination. The synergistic effect of valproate and ethosuximide supposed in that article may also be explained by the two drugs having a different mechanism of action.

Both Tallarida (1992) and Berenbaum (1989) advise to analyze drug interactions with the isobologram method. The problem with this method is that it only visualizes the interaction and that no statistical inference is given. Bourgeois used a method to quantify the difference between mono- and polytherapy, namely, the fractional effective concentration (FEC). The FEC is the ratio between the concentration of a drug in combination with another drug and the concentration at which the drug alone achieves the same effect. When the two FEC values of the two drugs are added, the FEC index is obtained. An additive interaction exists if the FEC index is between 0.7 and 1.3. If the FEC is below 0.7, there is supra-additivity, and an FEC over 1.3, indicates infra-additivity. This method quantifies the isobologram method but still does not use statistics to prove interaction because the border values are arbitrary and do not take into account the variance of the measurements. By calculating an expected regression curve of the combination therapy, we create points with variance that can be compared with actually measured points using statistical evidence by 95% CI, which is analogous to statistical testing with a P value of 0.05. Woolverton and Balster (1981) used linear regression by using only the linear portions of the dose-effect curves and they also determined 95% CI. Another advantage of our analysis is that we used

**TABLE 3**

<table>
<thead>
<tr>
<th></th>
<th>Valproate</th>
<th>Ethosuximide</th>
<th>Experimental Combination</th>
<th>Theoretical Additive</th>
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<tbody>
<tr>
<td>TD50 (mg/kg)</td>
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<td>−4.2−−0.5</td>
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**TABLE 4**

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<td>1.4–2.8</td>
<td>1.3–4.1</td>
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nonlinear regression. By doing this, it is not only possible to say something about the middle of the curve, where the TD$_{50}$ is located, but also to extrapolate with reasonable accuracy to the extremes of the curves, for example, the TD$_{10}$. In future experiments when therapeutic effect is measured, this might enable us to calculate the TD$_{10}$/ED$_{90}$ ratio. The TD$_{10}$/ED$_{90}$ and TD$_{50}$/ED$_{50}$ are not necessarily equal and the former is clinically more relevant because you want to obtain maximum therapeutic strength with minimum toxicity.

Our finding of infra-additivity for sedation when using a combination of valproate and ethosuximide compared with equal drug loads of valproate or ethosuximide monotherapy suggests that advantages may exist in combining these two drugs in low dosages. This calls for future experiments to measure both the therapeutic and toxic effects of this combination. The methodology used in this experiment may very well be used to test other combinations in the search for rational polytherapy. Such experiments may be used to identify those antiepileptic drug combinations that show enough promise to be tested in controlled clinical trials.

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References


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