Proc. Natl. Acad. Sci. USA
Vol. 89, pp. 11466–11470, December 1992
Medical Sciences

Methodologic aspects of a population pharmacodynamic model for cognitive effects in Alzheimer patients treated with tacrine

N. H. G. HOLFORD*† AND KARL E. PEACE‡

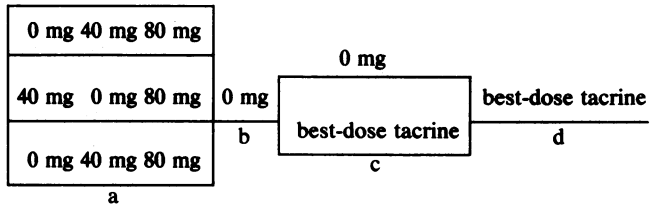
*Department of Pharmacology and Clinical Pharmacology, University of Auckland, Private Bag, Auckland, New Zealand; and ‡Biopharmaceutical Research Consultants, Inc., 4600 Stein Road, Suite B, Ann Arbor, MI 48105

Communicated by Pedro Cuatrecasas, July 16, 1992 (received for review May 8, 1992)

ABSTRACT **Tacrine is a cholinesterase inhibitor with activity in the central nervous system originally marketed for the reversal of competitive neuromuscular blockade. Because a marked reduction in cholinergic neurons is a hallmark of brain changes in Alzheimer disease, tacrine has been studied in two placebo-controlled clinical trials of patients with probable Alzheimer disease. Standard analysis of variance (ANOVA) and analysis of covariance (ANCOVA) have shown a difference between the tacrine group and the placebo group in terms of the cognitive component of the Alzheimer disease assessment scale at the end of the placebo-controlled phase. Due to limitations of ANOVA and ANCOVA, only a selected group of patients could be analyzed by those methods. A population pharmacodynamic model has been developed that allows the use of all observations from one or more trials to be combined. It can incorporate any sequence of active or placebo treatments and account for carryover effects of both placebo and active drug. The time courses of active or placebo treatment response and the development of tolerance to active drug or placebo can be defined. The model describes disease progression without treatment, the placebo effect, and the effect of tacrine as a function of daily dose. Placebo effect and active drug effects are modeled by effect site concentration components.**

Tacrine is a cholinesterase inhibitor with activity in the central nervous system originally marketed for the reversal of competitive neuromuscular blockade (1). A marked reduction in cholinergic neurons is a hallmark of brain changes in Alzheimer disease and has led to a search for therapies based on changing cholinergic function (2). In 1986 Summers *et al.* (3) reported an improvement in patients with Alzheimer disease who were treated with tacrine in doses up to 200 mg/day. This led to a large scale effort, sponsored by the National Institute on Aging, the Alzheimer's Disease and Related Disorders Association, and Parke–Davis Pharmaceutical Research Division of Warner Lambert Company, to evaluate the potential benefits of tacrine. It was soon realized that doses of tacrine >80 mg/day were associated with elevations in alanine aminotransferase and clinical trials at higher doses were curtailed by the Food and Drug Administration (4).

A modified protocol was developed (see below) that involved an initial randomized titration (enrichment) phase (*a*) followed by a placebo baseline washout phase (*b*), a double-blind phase that compared placebo with tacrine at the best tolerated dose in patients who reached a predefined response (*c*), and a blinded sustained active phase with all patients at their best dose of tacrine (*d*)



All doses are expressed as the total daily dose. Patients were to take the daily dose divided into four equal parts every 6 hr.

The primary measure of disease status was the cognitive component of the Alzheimer disease assessment scale (ADASC; ref. 5). Two large multicenter trials based on this new design have been completed: protocol 970-01 (6) and protocol 970-04 (7). Analysis of the double-blind phase of the 970-01 trial led to the conclusion that there was a significant difference between tacrine and placebo with a mean difference of 2.5 ADASC units after 6 weeks of active treatment at the best tolerated dose (40 or 80 mg/day) (8). This difference is ≈10% of the mean ADASC score at the start of the trial and the disease progresses at ≈6 units/year.

The efficacy of tacrine in these studies was assessed by standard analysis of variance (ANOVA) and covariance (ANCOVA) techniques. Due to the complexity of the trial protocol, only a subset of all treated patients were evaluated for efficacy by these techniques—i.e., patients who participated in the placebo-controlled double-blind phase—and then only over that phase. Of a total of 909 patients in both trials with an average of 5.8 assessments of ADASC per patient, only 400 patients were included in these efficacy analyses. Although ANCOVA and ANOVA techniques are based on linear fixed effects models, they are directed toward detecting randomized group differences—with and without adjustment for covariates. Questions that could not be answered by these techniques include (*i*) was the benefit proportional to dose, (*ii*) what is the effect of tacrine in the entire population studied for as long as they were studied, and (*iii*) how long did the effect of tacrine take to appear and how long did it last.

We describe a regression model for the data from these trials that is nonlinear and contains both fixed and random (mixed) effects. Parameters of the model are estimated by using the NONMEM software package of Beal and Sheiner (9). A feature of NONMEM is that information from each patient contributes to the estimation of model parameters even though all parameters may not be identifiable in each patient. This approach has allowed us to pose the above questions, as well as others, in quantitative form and address them to the

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: ADASC, Alzheimer disease assessment scale; MLE, maximum-likelihood estimation.

†To whom reprint requests should be addressed.

observations of these trials both separately and in combination. The general applicability of this approach has recently been reviewed (10).

Clinical Studies

Details of participating centers and the trial procedures are described elsewhere (6, 7). In brief, patients with a diagnosis of probable Alzheimer disease were assessed by ADASC at a screening visit and were subsequently entered into the titration phase, usually within 4 weeks of the first assessment. Each patient was randomly assigned to one of three treatment sequences (*i*) placebo, tacrine at 40 mg/day, tacrine at 80 mg/day; (*ii*) tacrine at 40 mg/day, tacrine at 80 mg/day, placebo; (*iii*) tacrine at 40 mg/day, placebo, tacrine at 80 mg/day. Because of concerns about toxicity from higher doses of tacrine, it was decided not to use 80 mg/day without a preceding 40 mg/day treatment. After 2 weeks at each titration step, the patient was assessed and the next treatment in the sequence was started. At the end of the third titration period, a 2-week blinded placebo baseline phase commenced. During this phase, the response in the three titration periods was evaluated and patients who improved by at least 4 units using the total ADASC on tacrine, in comparison to placebo, were assigned a "best" dose—i.e., either 40 or 80 mg/day. Patients with a best dose were then randomized to either a tacrine group or a placebo group and were followed in double-blind fashion for 6 weeks. Patients in the tacrine group were treated with their best dose from the titration phase. Then the patients in the tacrine group and the placebo group were treated for up to 6 weeks with the best dose of tacrine (sustained active phase) but were blinded to the actual dose.

Overview of Modeling Process

Because of the complexity of the model building and parameter estimation process, we provide a short background review of the process.

When modeling data reflecting some biological phenomenon, one postulates a relationship between the data and certain explanatory variables. A relationship may be symbolized as $y = f(\mathbf{x}; \boldsymbol{\theta}) + e$, where y is the data, $\mathbf{x}$ is a vector of explanatory variables, which may include experimental design features as well as covariates, $\boldsymbol{\theta}$ is the corresponding vector of unknown parameters, and e represents residual error. Residual error in this model is the total of all errors including measurement error, intersubject variation, and structural model misspecification error. For some experiments, a design may be used that will enable one to estimate variation in the measurement process and to test for model misspecification. This symbolization reflects residual error to be additive. In some applications, residual error may be multiplicative. The term $f(\mathbf{x}; \boldsymbol{\theta})$ is often referred to as the structural component of the model. Its form (polynomial, exponential, etc.) will be primarily driven by the biology of the phenomenon and what is empirically known about the relationship between y and $\mathbf{x}$.

When fitting a model to data, some method is used that permits the parameter vector $\boldsymbol{\theta}$ to be estimated as a function of the data. Common methods of estimation are least squares and maximum likelihood. Each uses a criterion of fit. For least-squares estimation, the criterion is to find values of the parameters that minimize the sum of squared deviations of the data about the structural component. For maximum-likelihood estimation (MLE), the criterion is to find the values of the parameters that maximize the probability (or likelihood) associated with the data. The criterion of fit can be translated into an objective function, which for MLE is most often the natural logarithm (ln) of the likelihood function (LF). The estimates of the parameters are then those values that optimize (minimize or maximize) the objective function.

If each component of $\boldsymbol{\theta}$ appears linearly in $f(\mathbf{x}; \boldsymbol{\theta})$, the estimation is said to be linear; otherwise it is considered nonlinear. As Beal and Sheiner (9) point out, before the availability of NONMEM, statistical software for nonlinear estimation permitted only a single source of residual variation. In modeling applications in the biological sciences, particularly in pharmacology and pharmacokinetics, often the data set one is trying to model has more than one source—e.g., within and between patient components. A model that expresses both sources could be written as: $y = f(\mathbf{x}; \boldsymbol{\theta}) + g_1(\mathbf{x}; \boldsymbol{\theta})e_1 + g_2(\mathbf{x}; \boldsymbol{\theta})e_2$, where e_1 and e_2 are the sources of variation and g_1 and g_2 may also be functions of the explanatory variables and unknown parameters. If the components of $\boldsymbol{\theta}$ enter $f(\mathbf{x}; \boldsymbol{\theta})$ nonlinearly, then the model is a nonlinear mixed (fixed and random) effects model; hence, the acronym NONMEM. This is the type of model that can be fit by using the NONMEM software package. Strictly speaking, the functions g_1 and g_2 should be linear in the parameters, but departures can be addressed by using approximating linear functions.

Fundamentally, this is the type of model we develop and fit to the ADASC data from the clinical studies of tacrine. However, there is no simple functional representation for $f(\mathbf{x}; \boldsymbol{\theta})$. Implicitly, $f(\mathbf{x}; \boldsymbol{\theta})$ is a composite (link) of the effect of placebo treatment and several structural forms. These forms incorporate pharmacokinetic and pharmacodynamic features of the dosing regimens in the trials using the notion of an effect site concentration.

Once a model (full) with n parameters has been fit by the MLE method, one can quantitatively assess the significance of a single parameter or of a combination (q) of parameters by likelihood ratio statistical theory. To do so, one refits the model (reduced) with all parameters in the particular group fixed at hypothesized values. Twice the difference between the values of ln(LF) for the full model and the reduced model is distributed approximately as χ^2 with q degrees of freedom. Parenthetically, this difference is often called the change in objective function. If $q = 1$, this difference would have to be 3.841 or larger to declare a single parameter as statistically significant at the 0.05 level. If $q = 2$, the critical value would be 5.991.

Model Development

Placebo Treatment. It is straightforward to define treatment with active medication, but the definition of placebo treatment is not as clear. Although patients were blinded to the actual treatment used throughout the trial, from the start of titration to the end of the sustained active phase, they were aware of a change in treatment because the protocol called for the number of tablets taken per day to be reduced and then built up over the next 2 days. We have therefore considered every change of treatment to be associated with a nominal placebo dose. This is assumed to be given every 2 weeks, including the start of the titration phase, except for weeks 2 and 4 of the double-blind placebo-controlled phase and sustained active phase (treatment was maintained for 6 weeks without change during these phases). Thus, we propose that a patient will have a placebo response every time he/she is aware that a treatment change has taken place. Any change in the setting of a blinded placebo-controlled crossover trial could mean an active treatment period has just started.

An instantaneous input of the placebo dose (bolus) is proposed because this is the simplest way to describe addition of placebo to the system. The shape of the resulting (hypothetical) placebo concentration time course allows flexibility in defining the time of the maximum placebo effect and the duration of the response.

Pharmacokinetic and Pharmacodynamic Models. Models were developed by using the notion of an effect site concentration (11). This allows effects of tacrine dosing to be delayed with respect to changes in plasma concentration, but at steady state it is assumed that the effect site concentration is equal to the average plasma concentration and proportional to the daily dose. The delay is modeled in terms of an equilibration half-time with the property of describing the exit rate of drug from a pharmacokinetic compartment. The physical interpretation of the equilibration half-time is not restricted to the kinetics of tacrine itself, but it can be explained by the kinetics of some physiological mediator of the eventually observed therapeutic effect (12).

In the absence of plasma concentration measurements sufficient to model the pharmacokinetics of tacrine, it is assumed that the average plasma concentration is instantaneously proportional to the daily tacrine dose. Furthermore, it is assumed that tacrine clearance is unaltered throughout the course of treatment, although it is possible to modify the predicted concentration by scaling the nominal clearance based on potential predictors of clearance such as weight, measured concentration, or renal function. Tacrine is not eliminated in any significant amount in the urine (13), so renal function is not expected to predict tacrine concentrations *per se* but may predict the existence of an unidentified metabolite eliminated by the kidneys, which may have pharmacological effects.

The model for placebo response is developed in terms of a hypothetical placebo concentration at the effect site. The placebo dose is modeled as if it were given instantaneously at the start of each treatment period—i.e., titration periods *i*, *ii*, and *iii*, double-blind placebo, double-blind best-dose phase, and sustained best-dose phase.

For simplicity it is assumed that the rate of disease progression is a linear function of time (at least over the period of the trial). Disease status at baseline S_o, and subsequent progression is predicted by $S(t)$, where $S(t) = S_o + \alpha \cdot t$, and where α is the rate of disease progression.

The pharmacodynamic (PD) model for the effect of active drug or placebo treatment may be a linear function of the effect site concentration (Ce) [e.g., $\mathrm{PD}(Ce) = \beta \cdot Ce$] or nonlinear [e.g., $\mathrm{PD}(Ce) = E_{max} \cdot Ce/(EC_{50} + Ce)$, where E_{max} is the maximum possible effect and EC_{50} is the concentration of Ce producing 50% of E_{max}].

The effect of treatment can be explained in terms of a change in the progression rate parameter, α, dependent on active drug effect site concentration, Ce_a: $S(t) = S_o + \alpha \cdot \mathrm{PD}(Ce_a) \cdot t$. A physical interpretation of this model might be to propose that tacrine slows the degeneration of some essential physiological substrate—e.g., cholinergic neurons. We call this the slope model.

However, an anticholinesterase such as tacrine may act more directly by restoring cholinergic transmission to a more functional level and thus produce a shift in the disease progression curve: $S(t) = S_o + \alpha \cdot t + \mathrm{PD}(Ce_a)$. Note that if the sign of β (or E_{max}) in the pharmacodynamic model is negative, the effect is equivalent to a shift of the curve to the right—i.e., postponing the time to reach the same extent of progression. We call this the offset model.

The time course of disease status can now be defined by the combination of disease progression, placebo response (predicted by Ce_p) and active treatment response (predicted by Ce_a). These separate influences are assumed to be independent and additive:

$$S(t) = S_o + \alpha \cdot t + \underset{\text{Active drug}}{\mathrm{PD}(Ce_a)} + \underset{\text{Placebo}}{\mathrm{PD}(Ce_p)}.$$

The effect site concentration is based on different models for the time course of concentration of tacrine, placebo, and a tolerance factor that might influence the response to tacrine. The concentration of tacrine (or a physiological mediator of the observed clinical response) at the site of action is assumed to be proportional to the daily dose. The time course of active substance at the effect site, $Ce_a(t)$, is predicted by

$$Ce_a(t) = Css_a \cdot [1 - \exp(-K_{eq_a} \cdot t)],$$

where Css_a is the steady-state plasma concentration of active substance, which has been maintained for time t. In general, it has been assumed that $Css_a = D/CL$, where D is the daily dose of tacrine and CL is tacrine clearance (see below). K_{eq_a} is the equilibration rate constant for the active substance, which is related to the equilibration half-time, $T_{eq_a} = \ln(2)/K_{eq_a}$.

The placebo concentration is assumed to be transient. It has been modeled by the time course of a substance administered as a bolus at the start of placebo treatment. The disappearance of placebo from the body is modeled by a hypothetical elimination half-time, T_{el_p}. A delay in onset of the placebo response can be modeled as if the placebo had entered an effect site with an equilibration half-time, T_{eq_p}, controlling the time course of placebo concentration at the effect site, $Ce_p(t)$:

$$Ce_p(t) = C_{o,p} \cdot K_{eq_p}/(K_{eq_p} - K_{el_p}) \cdot [\exp(-K_{el_p} \cdot t) - \exp(-K_{eq_p} t)],$$

where K_{eq_p} and K_{el_p} are related to the corresponding half-times of equilibration and elimination, T_{eq_p} and T_{el_p}. $C_{o,p}$ is the instantaneous concentration of placebo and is proportional to the nominal placebo dose. It has a value of 1 after the first placebo dose.

The development of tolerance has been modeled by proposing that a hypothetical antagonist factor is formed in proportion to exposure to tacrine. The pharmacokinetic model for the concentration of this factor (C_{tol}) is similar to the model discussed earlier for the effect compartment concentration of active substance:

$$C_{tol}(t) = Css_a \cdot [1 - \exp(-K_{eq_{tol}} \cdot t)],$$

where C_{tol} is formed in proportion to the average steady-state concentration of the active agent (Css_a) and is eliminated with a half-life, T_{tol}, with corresponding rate constant, $K_{eq_{tol}}$.

Initially, it might be assumed that the placebo contribution to the overall response is identical at the start of each treatment period. However, it is possible that the placebo response wanes with time. Diminishing placebo response has been modeled as an exponential decrease in the size of the apparent placebo dose administered at the start of each treatment period: Dose P_{tol} = Dose $P \cdot \exp(-K_{tol_p} \cdot t)$, where K_{tol_p} is a rate constant derived from a placebo tolerance half-life (T_{tol_p}), t is the time that has elapsed since the start of the first titration period, and Dose P is the first placebo dose.

The interaction of the tolerance factor with active drug is modeled by an influence on the active drug potency parameter, β_a:

$$f_{tol} = C_{tol}/(C_{tol} + TOL_{50}),$$

$$\beta_{a,tol} = \beta_a \cdot f_{tol},$$

where f_{tol} is the fraction of nontolerant effect produced by the tolerance factor. TOL_{50} is the concentration of C_{tol} that reduces f_{tol} to 0.5. It is assumed that the tolerance factor interacts instantaneously as soon as it is formed. This kind of model has been used to model the development of tolerance to nicotine (14).

The potential placebo response associated with starting a new active treatment period was modeled by an additional parameter, ADDP, which multiplies the predicted placebo response associated with active treatment. When ADDP = 0 the placebo effect of active treatment is 0 and when ADDP = 1 the placebo effect contributing to the total active treatment response is the same as the effect of placebo treatment alone. If ADDP is >1 this suggests that the placebo contribution to the total active treatment response is potentiated in comparison with treatment with placebo alone.

The concentration at the effect site has been predicted from the sum of the concentrations predicted from the current treatment and previous treatments. The effect site concentrations of active drug or placebo were predicted and summed separately. The average plasma concentration of tacrine from previous doses is assumed to go to 0 immediately when a new treatment is started. This is a reasonable assumption given a typical plasma elimination half-life of tacrine of 1.6 hr (13). The effect site concentration from previous tacrine treatments is assumed to disappear according to an exponential process with half-life T_{eq_a}. The effect site concentration of previous placebo treatments will be controlled by the more complex model shown above involving T_{eq_p} and T_{el_p}. The concentration of tolerance factor disappears in a fashion similar to that of the effect site concentration of tacrine but with half-time T_{tol}.

Detection of Population Differences. When more than one trial is available, it is possible to determine the similarities and differences between the populations represented in each trial. We have done this by incorporating a protocol scale factor into the model. This factor multiplies any of the previously described parameters of the model and is treated as an additional model parameter. In the case of the two tacrine trials, we assigned a fixed value of 1 to the protocol scale factor for the 970-01 protocol patients who were drawn from a United States population. The French patients in the 970-04 protocol had the scale factor estimated so that a model parameter—e.g., β_p, the placebo potency—for the French population could be different from the United States population. If the protocol scale factor is not distinguishable from 1, then one can conclude that the populations share the same value for the model parameter being tested.

Parameters and Estimation

Parameters. The main structural model parameters that were estimated are S_o, baseline disease status; α, disease progression rate; β_a, tacrine potency; β_p, placebo potency; T_{eq_a}, tacrine equilibration half-time; T_{eq_p}, placebo equilibration half-time; T_{el_p}, placebo elimination half-time; and T_{tol_p}, placebo tolerance half-time. A parameter describing the rightward shift of the disease progression curve (delay) was derived from the disease progression rate and tacrine potency. Additional parameters, which were estimated to test special models, are ADDP, active placebo factor; T_{tol_a}, active drug tolerance factor half-time; TOL_{50}, active drug tolerance factor potency; E_{max_a}, maximum active drug effect; and EC_{50_a}, active drug potency. Including the screening measurement of disease status, there were up to 10 measurements of disease status in each patient. All model parameters are not identifiable in individual patients who did not enter the double-blind comparison phase. However, the use of a population-based pharmacodynamic model potentially allows the use of information from all individuals in estimating the model parameters.

The variability of the structural model parameters may also be estimated. Two models for population variability were examined: (*i*) A proportional error model for the variability of the structural parameter estimates has been used, which implies that the variability in each estimate arises from a log normal distribution; and (*ii*) an additive error model has been used, which implies a normal distribution.

Different structural models or similar models with one or more parameters fixed can be compared by using the objective function value reported by NONMEM. A decrease in the objective function of 3.841 (equivalent to the 95% quantile of a χ^2 distribution with 1 degree of freedom) or more with the addition of a single parameter is significant at the 0.05 level (15). The variability of the parameter estimates in the population is shown as a coefficient of variation. It is calculated as the square root of the diagonal element of the NONMEM omega matrix of covariance estimates. The standard errors of the estimates and their coefficients of variation are obtained from the NONMEM standard error matrix.

Computational Methods. The parametric pharmacodynamic model described above was expressed as a code for the NMTRAN/NONMEM system (9). This permits us to estimate the parameters of a nonlinear mixed effects model and their variability. NMTRAN (version I, level 1.1) and NONMEM (version III, level 1.2) were executed on a Sun 4/330 computer. This is the most recent version of the software that has been distributed to NONMEM licensees. It should be recognized that this version uses a first-order approximation that has been shown to produce biased parameter estimates in some settings (16). Simulations of model predictions were performed with MKMODEL (17).

Simulation. Estimates of the model parameters are used to simulate the offset model to enable the reader to visualize predictions from the model (Fig. 1). The time course of ADASC is shown for two hypothetical treatment sequences. The upper curve is a patient treated with placebo alone. The lower curve is a patient treated with both placebo and tacrine at the doses shown. For clarity, placebo tolerance has not been included in the model. The net effect of tacrine is shown by the difference between the two curves.

The simulation shows the gradual increase in ADASC due to disease progression before the start of the placebo period of the titration phase at time 0. Because of the slow disappearance half-time of placebo in relation to the dosing interval of 2 weeks, it is possible to see an accumulation of placebo effect until the end of the double-blind placebo phase. There is then a 6-week period before the next placebo dose is given at the start of the sustained best-dose phase so that the placebo effect component wanes. The disappearance of the placebo effect is then seen after the start of the sustained best-dose phase, which approaches the predicted disease state in an untreated patient at ≈20 weeks.

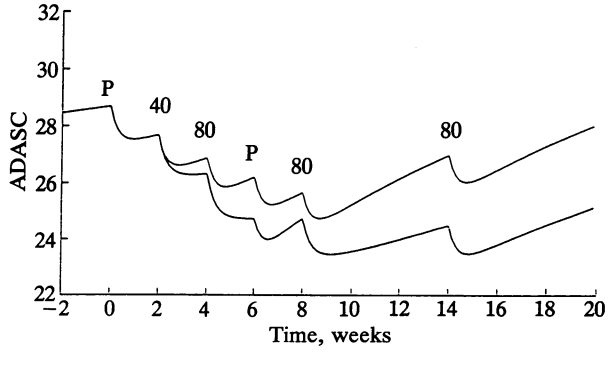


FIG. 1. Simulation of the time course of ADASC following two hypothetical treatment protocols. Upper curve, response to treatment with placebo; lower curve, response to treatment with tacrine at the daily dose rates shown. Dose labels of P, 40, and 80 mark the start of each treatment period. For example, at the beginning of week 8, 80 implies an active dose of 80 mg/day for the tacrine curve and a placebo (P) dose of 80 mg/day for the placebo curve.

Conclusion

The pharmacodynamic model presented allows the proportionality between dose and response to be assessed by using a linear or a nonlinear function. It allows all the observations from patients in the study to be included because it contains components that account for the varied treatments and recognizes the correlation of responses within individuals so that it can account for the changing numbers of participants in the trials. Finally, the incorporation of a kinetic component in the model allows a description of the time course of onset of action, whether due to active drug or placebo, and subsequent disappearance of the effect when treatment is stopped.

Encouragement from Dr. Carl Peck, Center for Drug Evaluation and Research, Food and Drug Administration, is gratefully acknowledged. We also respectfully acknowledge the investigators for the studies: 970-01, Ken Davis, M.D., Principal Investigator, and Leon Thal, M.D., Co-Principal Investigator for the National Institute on Aging sponsored multicenter clinical trial and the Tacrine Collaborative Study group; 970-04, Françoise Forette, M.D., Principal Investigator for the French Tacrine Study group. This research was sponsored by a grant from Parke-Davis Pharmaceutical Research Division, Warner Lambert Company.

1. Martindale, W. (1967) *The Extra Pharmacopoeia* (Pharmaceutical Press, London), 25th Ed.
2. Drachman, D. A. (1983) in *Alzheimer's Disease*, ed. Reisber, B. (Free Press, New York), pp. 340–345.
3. Summers, W. K., Majovski, L. V., Marsh, G. M., Tachiki, K. & Kling, A. (1986) *N. Engl. J. Med.* **315**, 1241–1245.
4. Gamzu, E. R., Thal, L. J. & Davis, K. L. (1990) *Adv. Neurol.* **51**, 241–245.
5. Rosen, W. G., Mohs, R. C. & Davis, K. L. (1984) *Am. J. Psychiatry* **141**, 1356–1364.
6. Department of Health and Human Services, Public Health Service, Food and Drug Administration (1991) *Peripheral and Central Nervous System Drugs Advisory Committee, March 15, 1991* (Transcript: Miller Reporting Company, 507 C Street, N.E., Washington, DC 20002).
7. Department of Health and Human Services, Public Health Service, Food and Drug Administration (1991) *Peripheral and Central Nervous System Drugs Advisory Committee, July 15, 1991* (Transcript: Miller Reporting Company, 507 C Street, N.E., Washington, DC 20002).
8. Davis, K. L., Thal, L. J., Ganzu, E., Davis, C. S., Woolson, R. F., Gracon, S. I., Drachman, D. A., Schneider, L. S., Whitehouse, P. J., Hoover, T. M., Morris, J. C., Kawas, C. H., Knofman, D. S., Earl, N. L., Kumar, V., Doody, R. S. & the Tacrine Collaborative Study Group (1992) *N. Engl. J. Med.* **327**, 1253–1259.
9. Beal, S. L. & Sheiner, L. B. (1980) *Am. Stat.* **34**, 118–119.
10. Rowland, M. & Aarons, L. (1992) *New Strategies in Drug Development and Clinical Evaluation: The Population Approach* (Commission of the European Communities, Brussels), Rept. EUR 13775.
11. Holford, N. H. G. & Sheiner, L. B. (1981) *Clin. Pharmacokinet.* **6**, 429–453.
12. Holford, N. H. G. (1990) in *Advanced Concepts of Pharmacokinetics and Pharmacodynamics*, ed. D'Argenio, D. Z. (Plenum, New York), pp. 55–59.
13. Forsyth, D. R., Wilcock, G. K., Morgan, R. A., Truman, C. A. & Roberts, C. J. C. (1989) *Clin. Pharmacol. Ther.* **46**, 634–641.
14. Sheiner, L. B. (1989) *Clin. Pharmacol. Ther.* **46**, 605–615.
15. Boechmann, A., Sheiner, L. B. & Beal, S. (1990) *NONMEM Users Guide* (NONMEM Project Group, Univ. of California, San Francisco), Part 5.
16. Rodman, J. H. & Evans, W. E. (1991) in *Advanced Methods of Pharmacokinetic and Pharmacodynamic Systems Analysis*, ed. D'Argenio, D. Z. (Plenum, New York), pp. 177–183.
17. Holford, N. H. G. (1990) *MKMODEL: A Pharmacological Modelling Tool* (Biosoft, Cambridge, U.K.).