Small Animal Model for Evaluating Neuroleptic Potency

JACK M. ROSENBERG *x and CESAR A. LAU-CAM

Received January 22, 1979, from the *Department of Pharmaceutical Sciences, St. John's University College of Pharmacy and Allied Health Professions, Jamaica, NY 11439.* Accepted for publication July 31, 1979. *Present address: Arnold and Marie Schwartz College of Pharmacy and Health Sciences, Brooklyn, NY 11201.

Abstract □ The prolactin response in the rat is proposed as a convenient and inexpensive test system for the assessment of the clinical potency of neuroleptic agents. Times of maximum elevation ($p < 0.01$) of serum prolactin levels were determined by a one-way analysis of variance and the Newman–Keuls test for a period of 180 min following intraperitoneal administration of chlorpromazine hydrochloride, fluphenazine hydrochloride, perphenazine, prochlorperazine edisylate, and trifluoperazine hydrochloride (phenothiazines); haloperidol (a butyrophenone); chlorprothixene (a thioxanthene); loxapine succinate (a dibenzoxazepine); and molindone hydrochloride (a dihydroindolene). Maximum serum prolactin elevations occurred as follows: chlorpromazine hydrochloride, 30–90 min; chlorprothixene and trifluoperazine hydrochloride, 30–150 min; fluphenazine hydrochloride, 90–150 min; haloperidol, 90 min; loxapine succinate, 30 min; molindone hydrochloride, 30 and 60 min; and perphenazine and prochlorperazine edisylate, 30–180 min. For drugs that exhibited statistically indistinguishable maximum serum prolactin elevations at more than one sampling time, one of these times was selected arbitrarily for construction of a log dose–prolactin response curve. Log dose–serum prolactin curves were constructed at a time of maximum serum prolactin elevation for each drug. Thirty minutes was utilized for loxapine succinate and molindone hydrochloride, and 90 min was used otherwise. These curves were nearly parallel, and each had an excellent linear fit. Based on these curves, the ratio of the weights of the neuroleptic and chlorpromazine hydrochloride required to produce the same prolactin-stimulating potency was determined for each drug. This ratio was compared to the clinically accepted ratio of the weights of the neuroleptic and chlorpromazine hydrochloride required to produce the same antipsychotic potency. This technique demonstrated a close correlation between the prolactin-stimulating potencies of neuroleptics in rats and accepted antipsychotic potency relationships in humans.

Keyphrases □ Neuroleptic agents—model for evaluation of potency, prolactin response in rats □ Phenothiazines—model for evaluation of potency, prolactin response in rats □ Models—evaluation of neuroleptic potency, prolactin response in rats □ Prolactin response, rats—model for evaluation of neuroleptic potency

Prolactin, an anterior pituitary hormone in animals, is structurally similar to growth hormone (1). The dominant hypothalamic regulation of prolactin secretion in mammals appears to be inhibitory and under the control of prolactin-inhibitory factor (2). Prolactin-inhibitory factor is liberated by the hypothalamus into the portal vessels as a result of afferent dopaminergic impulses (3). It is unclear whether dopamine is a constituent of prolactin-inhibitory factor or is the factor itself (4).

Evidence of a prolactin-releasing factor exists, but its activity in controlling prolactin secretion clearly is less important than prolactin-inhibitory factor (5). Thyrotropin-releasing hormone also stimulates the secretion of prolactin- and thyroid-stimulating hormone, but thyrotropin-releasing hormone presumably does not play an important role in physiological prolactin regulation (5).

BACKGROUND

Enhanced prolactin secretion was reported following neuroleptic administration to humans (6, 7) and is associated with galactorrhea and other endocrine disturbances which may accompany the use of such drugs (8). A fairly good correlation between the antipsychotic potency of various neuroleptics and their human prolactin-stimulating response was found (6, 9, 10). A notable exception is clozapine, which does not seem to be a potent or consistent prolactin stimulator in humans; however, it does stimulate prolactin in rats (11, 12). Despite this exception, the prolactin response to a test dose of a new neuroleptic in normal human volunteers was proposed as a useful means to screen its efficacy and potency prior to clinical studies in humans (6, 10).

In addition to the various neurochemical techniques using animals to assess antipsychotic activity and/or potency (13–16), Clemens *et al.* (17) reported that when chlorpromazine and thioridazine, two phenothiazines of comparable antipsychotic potency, were given to rats, serum prolactin levels were stimulated to a similar extent. This observation was based on serum samples obtained 2 hr after drug administration. Meltzer *et al.* (18) demonstrated that the rank orders of average daily doses of nine neuroleptic agents in rat serum samples obtained 30 min after drug administration correlated with their corresponding doses needed to double serum prolactin levels. These correlation studies as well as other studies (11, 12) did not consider the possibly significant variation in serum prolactin profiles with respect to time for individual antipsychotic drugs and the effects of this parameter on observed magnitudes of prolactin stimulation. Furthermore, it was suggested that pituitary dopamine receptors that influence prolactin secretion in rats may differ significantly from those relevant to the antipsychotic action of neuroleptics (12).

A study was designed in rats to determine the times of maximum elevation of serum prolactin for representative antipsychotic drugs following their intraperitoneal administration and to investigate thoroughly the correlation between prolactin-stimulating potency of neuroleptics in rats and their clinical potency.

EXPERIMENTAL

In Stage 1 of this study, experiments were conducted to determine the times of maximum serum prolactin elevation for representative neuroleptics following their intraperitoneal administration. In Stage 2, experiments were conducted to determine the potency of each neuroleptic in inducing prolactin release at a time of maximum serum prolactin elevation.

Drugs—The following nine pharmacological agents, representing all five classes of antipsychotics approved for clinical use in the United States, were investigated: chlorpromazine hydrochloride[1], fluphenazine hydrochloride[2], perphenazine[3], prochlorperazine edisylate[1], and trifluoperazine hydrochloride[1] (phenothiazines); haloperidol[4] (a butyrophenone); chlorprothixene[5] (a thioxanthene); loxapine succinate[6] (a dibenzoxazepine); and molindone hydrochloride[7] (a dihydroindolone).

Animals—Male Sprague–Dawley rats[8], 225–300 g, were housed for 21 days in a temperature-controlled environment (23 ± 3°) which was illuminated artificially (lights on daily from 7:00 am to 7:00 pm). The animals were given food[9] and water *ad libitum*.

Times of Statistically Significant Maximum Serum Prolactin Elevation—Rats were divided into 11 groups of 48. The following antipsychotics were administered in equipotent dose equivalents for humans (19): chlorpromazine hydrochloride (10 mg/kg), chlorprothixene (10 mg/kg), fluphenazine hydrochloride (0.2 mg/kg), haloperidol (0.2 mg/kg), loxapine succinate (1 mg/kg), molindone hydrochloride (1 mg/kg), perphenazine (1 mg/kg), prochlorperazine edisylate (1.5 mg/kg), and trifluoperazine hydrochloride (0.5 mg/kg).

Solutions of these drugs were prepared to contain the indicated mil-

[1] Smith Kline and French, Philadelphia, Pa.
[2] E. R. Squibb & Sons, Princeton, N.J.
[3] Schering Corp., Kenilworth, N.Y.
[4] McNeil Laboratories, Fort Washington, Pa.
[5] Roche Laboratories, Nutley, N.J.
[6] Lederle Laboratories, Pearl River, N.Y.
[7] Endo Inc., Garden City, N.Y.
[8] Taconic Farms, Germantown, N.Y.
[9] Purina Lab Chow, Ralston Purina Co., St. Louis, Mo.

0022-3549/80/0100-0074$01.00/0

Table I—Prolactin-Stimulating and Therapeutic Potencies of Neuroleptics Relative to Chlorpromazine Hydrochloride

Neuroleptic	Prolactin-Stimulating Potency[a]	Antipsychotic Potency[b]
Chlorpromazine hydrochloride	1.0	1
Chlorprothixene	1.0	1–2.3
Prochlorperazine edisylate	5.7	6.2–7.7
Perphenazine	9.1	10–11.9
Molindone hydrochloride	9.3	5–19.6
Loxapine succinate	10.6	8–12
Trifluoperazine hydrochloride	33.3	20–35
Fluphenazine hydrochloride	62.5	50–90.9
Haloperidol	86.0	50–91

[a] Calculated from log dose–response regression curves (Figs. 4 and 5). [b] From Refs. 19, 24, and 26.

ligram-per-kilogram doses in 1 ml of physiological saline or, with haloperidol, chlorprothixene, and perphenazine, in 1 ml of 0.1 *M* tartaric acid to solubilize these agents. These solutions or equal volumes of vehicles were given intraperitoneally to experimental and control groups, each group consisting of eight subgroups of six animals each. Blood samples were collected by decapitation at 0, 30, 45, 60, 90, 120, 150, and 180 min after drug or vehicle administration.

Potency of Neuroleptics in Inducing Prolactin Secretion—Rats were divided into nine groups of 42. For each drug, six subgroups of seven rats were utilized. One animal in each subgroup served as a control. Every drug was administered in six serial dilutions. The concentrations in 1 ml of vehicle were: chlorpromazine hydrochloride and chlorprothixene, 25–0.781 mg/kg; fluphenazine hydrochloride, 1–0.031 mg/kg; haloperidol, 0.5–0.012 mg/kg; loxapine succinate and molindone hydrochloride, 1.25–0.078 mg/kg; perphenazine and prochlorperazine edisylate, 5–0.156 mg/kg; and trifluoperazine hydrochloride, 2–0.062 mg/kg.

Blood samples were obtained by decapitation at a statistically significant ($p < 0.01$) time of maximum serum prolactin elevation for each drug as determined by a one-way analysis of variance (20) and the Newman–Keuls test (21) from Stage 1 of this study.

Sample Collection and Assay—All decapitations were conducted between 2:00 and 4:00 pm. Blood was collected from the trunk portion and allowed to stand for 10 min at 4°; the serum was separated and frozen at −40° until hormone analysis could be performed. Serum samples were measured for prolactin using a radioimmunoassay kit[10]. The assays, using a double antibody radioimmunoassay, were performed according to the instructions.

The rat prolactin was iodinated by a modification of the Hunter–Greenwood method (22). Each serum sample was assayed in duplicate, and the average was taken as representative of the true prolactin concentration[11]. All test and control samples were determined concurrently for each drug. The intra- and interassay variations were 4.2 and 9.4%, respectively.

RESULTS

Time Course of Prolactin Response to Neuroleptics—Evaluation of the serum prolactin levels over 180 min following intraperitoneal injection of the various neuroleptic agents indicated that statistically significant ($p < 0.01$) maximum serum prolactin elevations occurred at the following times: chlorpromazine hydrochloride, 30–90 min; chlorprothixene and trifluoperazine hydrochloride, 30–150 min; fluphenazine hydrochloride, 90–150 min; haloperidol, 90 min; loxapine succinate, 30 min; molindone hydrochloride, 30 and 60 min; and perphenazine and prochlorperazine edisylate, 30–180 min (Figs. 1–3).

For drugs that exhibited statistically indistinguishable maximum serum prolactin elevations at more than one sampling time, one of these times was selected arbitrarily for each drug for subsequent experiments performed in Stage 2. Thirty minutes was utilized for loxapine succinate and molindone hydrochloride, and 90 min was used otherwise.

[10] National Institute of Arthritis, Metabolism, and Digestive Disease (NIAMDD) Rat Pituitary Hormone Distribution Program, National Pituitary Agency, Baltimore, Md.

[11] All results are expressed in terms of NIAMDD rat prolactin.

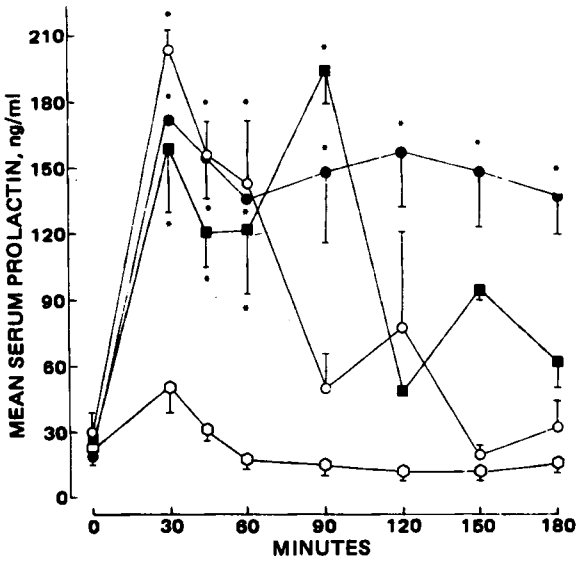

Figure 1—*Effect of intraperitoneally administered chlorpromazine hydrochloride (■), molindone hydrochloride (○), and prochlorperazine edisylate (●) on serum prolactin levels in male rats over 180 min. The control groups received normal saline (○). Each point is the mean for six animals. Vertical lines show the standard error of the means. Maximum serum prolactin elevations ($p < 0.01$) as determined by a one-way analysis of variance and the Newman–Keuls test are indicated by asterisks.*

Neuroleptic Dose–Prolactin Response Curves at Times of Maximum Serum Prolactin Elevation—Figures 4 and 5 show the log dose–prolactin response curves for the nine drugs studied as calculated by standard linear regression analyis (23). The slopes of the regression lines were: chlorpromazine hydrochloride, fluphenazine hydrochloride, haloperidol, and prochlorperazine edisylate, 1.9; chlorprothixene, loxapine succinate, and perphenazine, 1.8; and molindone hydrochloride and trifluoperazine, 1.6. The correlation coefficients for the log dose–prolactin curves were significant ($df = 4$) as follows: chlorpromazine, 0.91 ($p < 0.005$); chlorprothixene, 0.93 ($p < 0.005$); fluphenazine hydrochloride, 0.97 ($p < 0.005$); haloperidol, 0.95 ($p < 0.005$); loxapine succinate, 0.87 ($p < 0.01$); and prochlorperazine edisylate and trifluoperazine hydrochloride, 0.94 ($p < 0.005$).

Prolactin-Releasing Potency of Neuroleptics at Times of Maxi-

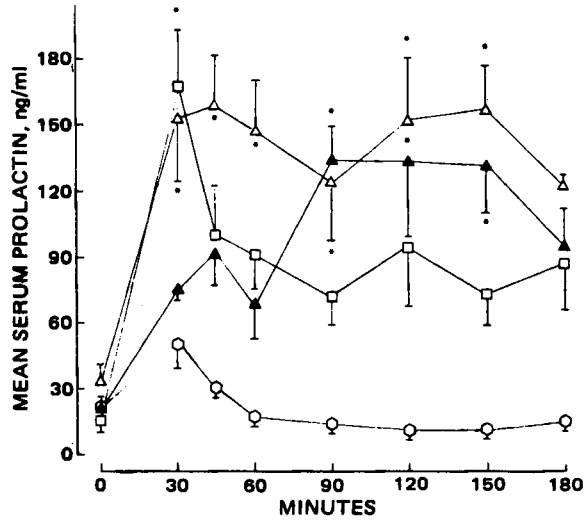

Figure 2—*Effect of intraperitoneally administered fluphenazine hydrochloride (▲), loxapine succinate (□), and trifluoperazine hydrochloride (△) on serum prolactin levels in male rats over 180 min. The control groups received normal saline (○). Each point is the mean for six animals. Vertical lines show the standard error of the means. Maximum serum prolactin elevations ($p < 0.01$) as determined by a one-way analysis of variance and the Newman–Keuls test are indicated by asterisks.*

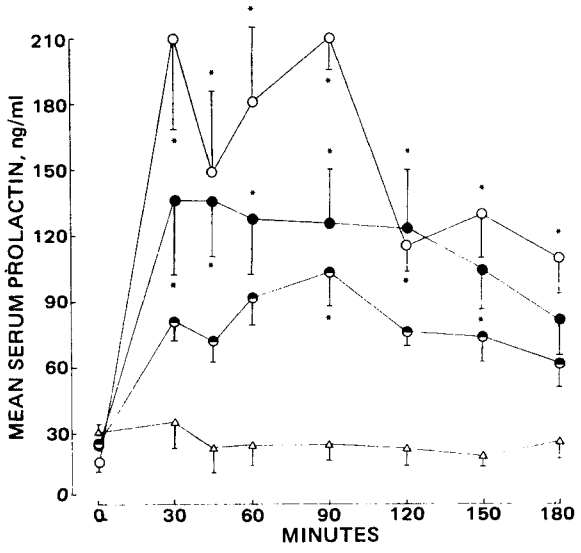

Figure 3—*Effect of intraperitoneally administered haloperidol (◓), chlorprothixene (●), and perphenazine (○) on serum prolactin levels in male rats over 180 min. The control group received 0.1* M *tartaric acid (△). Each point is the mean for six animals; vertical lines show the standard error of the means. Maximum serum prolactin elevations ($p < 0.01$) as determined by a one-way analysis of variance and the Newman–Keuls test are indicated by asterisks.*

mum Serum Prolactin Elevation—Doses (milligrams per kilogram) of neuroleptics calculated from log dose–response curves (Figs. 4 and 5) that were equipotent to 5.0 mg of chlorpromazine hydrochloride/kg in releasing prolactin are presented in Table I. In addition, the clinically defined therapeutic potencies of these drugs are compared on a weight to weight basis and related to their prolactin-stimulating potency. A commonly used chlorpromazine hydrochloride dose was selected as the reference because comparison of the activity of other antipsychotics to this neuroleptic is common (24–26).

DISCUSSION

Neuroleptic drugs belonging to three chemical groups, prochlorperazine, haloperidol, and thiothixene, produce parallel log dose–prolactin response curves in humans, presumably due to their antidopaminergic effects (10). When nine representative drugs belonging to five chemical classes of neuroleptics were administered to rats at experimentally determined times of maximum serum prolactin elevation [which do not vary

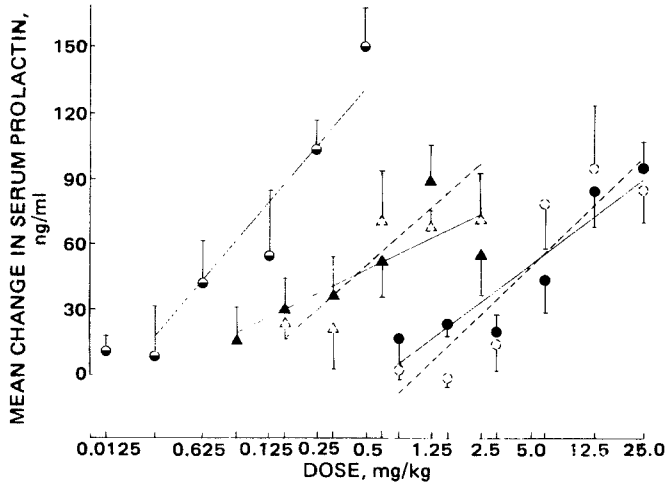

Figure 4—*Log dose–prolactin regression lines of chlorpromazine hydrochloride (○), chlorprothixene (●), haloperidol (◒), loxapine succinate (△), and molindone hydrochloride (▲). The y axis (mean change in serum prolactin) expresses the mean elevation of the prolactin concentration above the mean of the corresponding control group values. Each point represents the mean response of six experiments; vertical lines show the standard error of the means.*

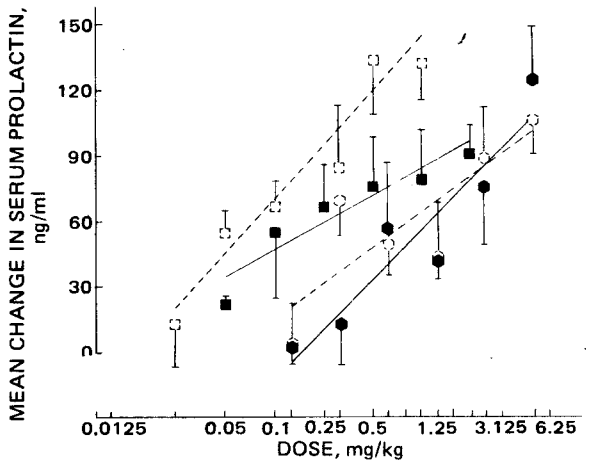

Figure 5—*Log dose–prolactin regression lines of fluphenazine hydrochloride (□), perphenazine (○), prochlorperazine edisylate (●), and trifluoperazine hydrochloride (■). The y axis (mean change in serum prolactin) expresses the mean elevation of the prolactin concentration above the mean of the corresponding control group values. Each point represents the mean response of six experiments; vertical lines show the standard error of the means.*

significantly for an individual neuroleptic in response to dose variations (6, 10)], nine nearly parallel log dose–prolactin response regression lines were calculated. The correlation coefficient for each drug was highly significant and indicated excellent linear fit. These results indicate that there also is a common mechanism for inducing prolactin release in rats, presumably due to their antidopaminergic effects.

To evaluate the prolactin-releasing potency of each drug at an experimentally determined time of maximum serum prolactin elevation, the log dose–prolactin response curves were utilized. Based on these curves, the calculated ratio of the neuroleptic to the chlorpromazine hydrochloride prolactin-stimulating potency was compared to its reported antipsychotic potency ratio. For seven of the nine drugs, the neuroleptic to chlorpromazine potency ratios in rats were consistent with the accepted neuroleptic to chlorpromazine potency relationships for humans. The prolactin potency ratios of perphenazine and prochlorperazine to chlorpromazine were only slightly below the accepted clinical potency relationships.

The data indicated a better relationship between the prolactin-releasing potency of neuroleptics in rats and the clinically accepted therapeutic potency in humans than was reported elsewhere in an animal system (18). For example, haloperidol was reported to be only 16.6 times more potent than chlorpromazine in inducing prolactin secretion using a fixed 30-min comparison of serum prolactin levels (18). For the experimentally derived 90-min maximum elevation of serum prolactin for haloperidol, which also was its peak prolactin response time observed in humans following intramuscular adminstration (10), a much better correlation was obtained between prolactin stimulation potency and the accepted clinical potency range (19, 24).

Due to the variability in the time–serum prolactin profiles of the individual neuroleptics following intraperitoneal administration, their potency and prolactin response ratios would have been altered if a fixed time comparison of all prolactin levels was made rather than a comparison at the time of each drug's maximum serum prolactin elevation. The different times for maximum elevation of serum prolactin observed for several neuroleptics in this study, as well as those cited by other investigators but involving humans (6), suggest a variation in the onset of action for different drugs due to their molecular characteristics. Future work should evaluate this possible relationship.

For the nine drugs studied, which represent all chemical classes of antischizophrenic agents approved for clinical use in the United States, there is evidence to suggest that clinically accepted neuroleptic potency relationships in humans closely correlate with prolactin-stimulating properties in male rats when measured at a time of maximum serum prolactin elevation. This technique may be a convenient alternative to the proposed use of normal volunteers (6, 10) as a screening test for establishing the potency of new neuroleptics.

REFERENCES

(1) A. G. Frantz and D. L. Kleinberg, *Science*, **170**, 745 (1970).

(2) M. T. Buckman and G. T. Peake, *J. Am. Med. Assoc.*, **236**, 871 (1976).
(3) I. A. Kamberi, R. S. Mical, and J. C. Porter, *Experientia*, **26**, 1150 (1970).
(4) E. Fluckiger, *Bull. Schweiz. Akad. Med. Wiss.*, **34**, 191 (1978).
(5) M. Szabo and L. A. Frohman, *Endocrinology*, **98**, 1451 (1976).
(6) E. J. Sachar, P. H. Gruen, N. Altman, F. S. Halpern, and A. G. Frantz, in "Hormones, Behavior, and Psychopathology," Raven, New York, N.Y., 1976, pp. 161–176.
(7) E. J. Sachar, P. H. Gruen, N. Altman, G. Langer, F. S. Halpern, and M. Liefer, in "Neuroregulators and Psychiatric Disorders," E. Usdin, D. Hamburg, and J. Barchas, Eds., Oxford University Press, New York, N.Y., 1977, pp. 242–249.
(8) R. Dickey and S. Stone, *Clin. Obstet. Gynecol.*, **18**, 95 (1975).
(9) H. Y. Meltzer and V. S. Fang, *Arch. Gen. Psychiat.*, **33**, 279 (1976).
(10) G. Langer, E. J. Sachar, F. S. Halpern, P. H. Gruen, and M. Solomon, *J. Clin. Endocrinol. Metab.*, **45**, 996 (1977).
(11) H. Y. Meltzer, S. Daniels, and V. S. Fang, *Life Sci.*, **17**, 339 (1976).
(12) H. Y. Meltzer, R. G. Fessler, and V. S. Fang, *Psychopharmacology*, **54**, 183 (1977).
(13) P. Seeman and T. Lee, *Science*, **188**, 1217 (1975).
(14) M. Goldstein, R. L. Bronaugh, T. Ohashi, and D. Eberstein, in "Neuroregulators and Psychiatric Disorders," E. Usdin, D. Hamburg, and J. Barchas, Eds., Oxford University Press, New York, N.Y., 1977, pp. 538–545.
(15) P. Seeman, M. Chou-Wong, J. Tadesco, and K. Wong, *Proc. Natl. Acad. Sci. USA*, **72**, 4376 (1975).
(16) S. H. Snyder, *Am. J. Psychiat.*, **133**, 197 (1976).
(17) J. A. Clemens, E. B. Smalstig, and B. D. Sawyer, *Psychopharmacologia*, **40**, 123 (1974).
(18) H. Y. Meltzer, D. J. Goode, and V. S. Fang, in "Psychopharmacology: A Generation of Progress," M. A. Lipton, A. DiMascio, and K. F. Killam, Eds., Raven, New York, N.Y., 1978, p. 509.
(19) American Medical Association Department of Drugs, "A.M.A. Drug Evaluations," Publishing Sciences Group, Littleton, Mass., 1977, pp. 444–448.
(20) B. J. Winer, "Statistical Principles in Experimental Design," 2nd ed., McGraw-Hill, New York, N.Y., 1961, p. 776.
(21) *Ibid.*, p. 191.
(22) W. M. Hunter and F. C. Greenwood, *Nature*, **194**, 495 (1962).
(23) B. J. Winer, "Statistical Principles in Experimental Design," 2nd ed., McGraw-Hill, New York, N.Y., 1961, p. 94.
(24) J. M. Davis, *Arch. Gen. Psychiat.*, **33**, 858 (1976).
(25) G. L. LaVigne and R. J. Baldessarini, *Am. J. Psychiat.*, **133**, 852 (1976).
(26) J. M. Davis, *Curr. Psychiatr. Ther.*, **17**, 209 (1977).

ACKNOWLEDGMENTS

Presented at the Natural Products and Medicinal Chemistry Section, APhA Academy of Pharmaceutical Sciences, Anaheim meeting, April 1979.

The authors thank the National Institute of Arthritis, Metabolism, and Digestive Disease Rat Pituitary Hormone Distribution Program and Dr. A. F. Parlow for their gift of radioimmunoassay materials. The authors also thank Dr. Howard McGuire, Long Island University, for assistance with the statistical analysis.

Further Considerations on Model-Independent Bioavailability Estimation

S. HWANG ˣ and K. C. KWAN

Received December 8, 1978, from *Merck Sharp & Dohme Research Laboratories, West Point, PA 19486.* Accepted for publication July 31, 1979.

Abstract □ Assumptions attendant to model-independent bioavailability estimation were reexamined. Particular attention was given to the situation where an intravenous reference is not available and nonrenal clearance is assumed to be constant between treatments. Under these circumstances, the previously proposed approximation was compared with other bioavailability estimators. On the basis of error analysis, a procedure was devised to yield optimal relative bioavailability estimates.

Keyphrases □ Bioavailability—model-independent estimation □ Drug availability—model-independent estimation □ Models—bioavailability estimation, equations

In a previous report (1), a model-independent method to assess bioavailability was suggested. The procedure calls for an initial determination of plasma clearance from an intravenous reference and assumptions concerning changes therein following the test dose(s). The proposed solutions are exact except when an intravenous reference is not available and nonrenal clearance is assumed to be unchanged between treatments. For this latter situation, an approximate solution was suggested initially with the support of a simulated example (1) and verified subsequently with experimental results (2).

This report provides a rigorous analysis of this approximation and the means to optimize its solutions. Its merits are examined relative to those of the dose-adjusted ratio of urinary recoveries of unchanged drug and of the area under the plasma concentration–time curve. It will be shown that where the nonrenal clearances are unchanged, the proposed approximation (1) is always superior to area ratios and often is better than urine ratios. Conditions under which relative bioavailability estimates should be optimal are discussed.

THEORETICAL

Bioavailability following a nonintravascular treatment, x, can be estimated by:

$$F^x = \frac{\dot{V}^x_{cl,p} AUC^x_\infty}{D^x} = \frac{\dot{V}^x_{cl,p} U^x_\infty}{D^x \dot{V}^x_{cl,r}} \qquad \text{(Eq. 1)}$$

where F is the fraction of the dose, D, absorbed; AUC_∞ is the total area under the plasma concentration–time curve; U_∞ is the total amount of unchanged drug excreted in the urine; and $\dot{V}_{cl,p}$ and $\dot{V}_{cl,r}$ are the plasma and plasma renal clearances, respectively. Except for $\dot{V}^x_{cl,p}$, the terms on the right side of Eq. 1 are known or can be calculated from plasma and/or urinary excretion data following treatment x.

On the other hand, plasma clearance must be determined by an independent experiment. Ideally, an intravenous tracer dose is administered concurrently with x such that the plasma clearance of the labeled drug becomes the estimate of $\dot{V}^x_{cl,p}$. An alternative solution was proposed (3, 4) whereby plasma clearance is estimated from separate treatments in which the renal drug clearance is perturbed in a controlled manner. The assumptions are that the perturbing influence on the kidney remains constant with time and that the same dose fraction is absorbed between

0022-3549/80/0100-0077$01.00/0