Bromperidol Radioimmunoassay: Human Plasma Levels

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Abstract □ A sensitive radioimmunoassay (RIA) procedure was developed to assay for bromperidol levels in human plasma after therapeutic drug administration. The antisera used in the RIA procedure was generated in rabbits against a haloperidol–bovine serum albumin conjugate. Tritiated haloperidol was used as the radioligand in the assay. A single ether extraction of alkalinized plasma was used to separate bromperidol from its more polar metabolites and to reduce assay variability encountered with a direct plasma assay. The lower limit of detection was ~0.5 ng/mL of parent drug in plasma. The assay exhibited within- and between-assay variabilities of ~9 and 14%, respectively. A 103–106% recovery of bromperidol from quality control plasma samples was observed over the concentration range of 1–150 ng/mL. A correlation coefficient of 0.9999 with respect to measured *versus* expected bromperidol content in the quality control plasma samples was exhibited. Cross-reactivity characteristics of the antisera indicated that dehydrobromperidol could significantly interfere (~25% cross-reactivity) with the RIA procedure. However, biotransformation studies have not suggested this compound as a metabolite of bromperidol. Predose (C_{min}) plasma levels of bromperidol in schizophrenic patients maintained on drug therapy are also reported.

Keyphrases □ Bromperidol—radioimmunoassay, human plasma levels, haloperidol-bovine serum albumin conjugate □ Radioimmunoassay—bromperidol, human plasma levels, haloperidol-bovine serum albumin conjugate

Bromperidol (I), 4-[4-(*p*-bromophenyl)-4-hydroxypiperidino]-4′-fluorobutyrophenone, the bromo analogue of haloperidol (II), is a potent neuroleptic agent which has been under clinical investigation for the treatment of psychoses such as schizophrenia. The bioavailability and pharmacokinetics of this drug in dogs under steady-state conditions following multiple-dose oral administration have been reported (1). A limited study comparing preliminary pharmacokinetic results from dogs and humans was also reported (2). In general, relating pharmacokinetics of the psychotropic agents with effective treatment of psychiatric disorders is difficult partly due to the lack of a sensitive and reliable analytical methodology. This problem can be aggravated by the different neuroleptic plasma levels observed in patients receiving concurrent medication (3).

A radioimmunoassay (RIA) method was developed in our laboratory and was used to measure levels of bromperidol in human plasma (4, 5). With this methodology the bioavailability and pharmacokinetics of bromperidol in patients chronically receiving drug therapy was studied (5). This paper describes the RIA procedure used to measure bromperidol plasma levels in schizophrenic patients. Also reported are mean C_{min} drug plasma levels from 15 schizophrenic patients maintained on various drug regimens.

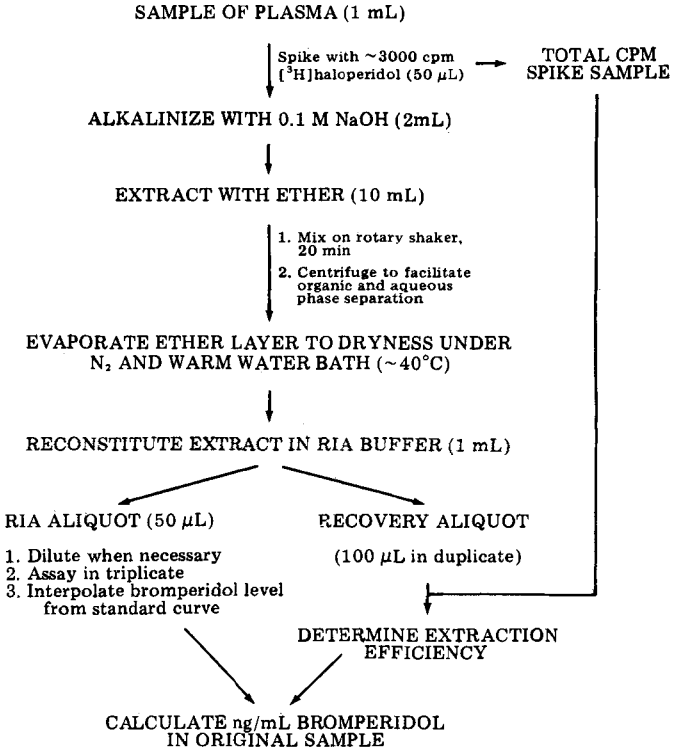
EXPERIMENTAL

Materials—Antisera[1] used in the RIA procedure was generated in rabbits against a haloperidol hapten chemically linked to bovine serum albumin *via* the tertiary alcohol functional group. Tritiated haloperidol[1], specific activity ~14 Ci/mmol, was used as the radioligand in the RIA procedure and to determine extraction efficiencies of drug material in the biological sample extraction procedure.

Radioimmunoassay—A bromperidol standard RIA curve was routinely generated from a series of test tubes (12 × 75 mm) prepared to contain an extract of control plasma sample (50 μL), unlabeled competitor (bromperidol, 0.02–10.0 ng in 50 μL of buffer), [^{3}H]haloperidol (50 μL containing ~6600 cpm), haloperidol antisera (50 μL, final antisera dilution of 1:1800), and RIA buffer (0.1 M sodium phosphate, 0.9% NaCl, 0.1% bovine IgG, and 0.1% sodium azide, pH 7.0) to achieve a final volume of 600 μL/tube. The contents were then mixed and incubated[2] at room temperature (~21°C) for ~18 h. (Note that this incubation period was chosen for convenience, since equilibrium of the radioligand binding to the antisera was demonstrated for an incubation period of 6 h.) Bound and free [^{3}H]haloperidol were separated by adsorption of free radioligand using dextran-coated charcoal (200 μL of a suspension containing 1 g of charcoal[2] and 100 mg of dextran[3] in 50 mL of RIA buffer). The charcoal suspension was incubated for 1.5 h, at room temperature. Centrifugation at ~330×*g* for 15 min adequately separated the charcoal-adsorbed material from that bound to the antisera. The supernatants were decanted into scintillation vials containing 15 mL of liquid scintillation cocktail[4] and 600 μL of water to aid dissolution of the sample. Bound radioactivity for each sample was determined using a liquid scintillation counter[5].

Quality control plasma samples were prepared to contain known amounts of bromperidol ranging from 1 to 150 ng/mL. These samples were prepared at the start of the study, stored at ~−20°C, and assayed throughout the study period (~6 months). Data from these analyses provided a measure of the drug stability in the biological matrix, as well as information from which assay statistics were calculated.

Patient plasma samples and quality control plasma samples were extracted according to the procedure outlined in Scheme I. This extraction procedure



Scheme I—Plasma sample extraction procedure and assay protocol for the bromperidol RIA.

[1] Janssen Pharmaceutica, Beerse, Belgium. The haloperidol contained one tritium atom *ortho* to the chlorine atom.

[2] Norite SG Extra; J. T. Baker Chemical Co., Phillipsburg, N.J.
[3] Dextran T-70; Pharmacia Fine Chemicals, Piscataway, N.J.
[4] Biofluor; New England Nuclear Corp., Boston, Mass.
[5] Model SL4000; Intertechnique Corp., IN/US, Fairfield, N.J.

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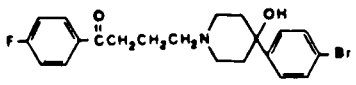
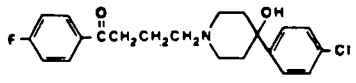
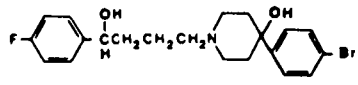
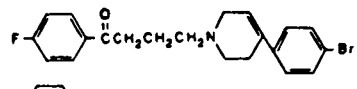
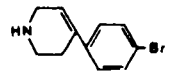
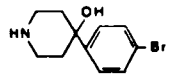
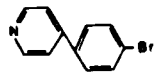
Table I—Compounds Examined for Cross-Reactivity Characteristics

Compound	Structure	D_{50}[a], ng	Relative Percent Cross-Reactivity[b]
I		0.26	100.0%
II		0.28	92.9%
III		277.4	0.1%
IV		1.07	24.3%
V		>1000	N.D.I.[c]
VI		>1000	N.D.I.
VII		5.76	4.5%
VIII		>1000	N.D.I.
IX		>1000	N.D.I.
X		>1000	N.D.I.

[a] D_{50} is the amount of compound required to depress the radioligand binding to the antisera 50%. [b] (D_{50} bromperidol/D_{50} competitor) × 100. [c] No detectable interference.

was required in order to remove potential interferences (presumably conjugates of bromperidol). The reconstituted extracts, or dilutions thereof, were assayed in replicate (3 × 50-μL aliquots) for drug content as described for the standard curve.

Data Processing—The standard curve and plasma bromperidol levels were determined using the logit-log computer program of Frazier and Rodbard (6).

Antisera Selectivity—The cross-reactivity of the compounds in Table I was determined by competing the individual substances, over a range of 0.05–1000 ng, with a fixed amount of radioligand and antisera. A 50% displacement value (D_{50}, amount of compound required to displace the radioligand binding to the antisera by 50%) was determined for each compound. The ratio of D_{50} observed for bromperidol to the D_{50} observed for the other compounds was used to determine a relative percent cross-reactivity.

Patient Samples—Schizophrenic patients (n = 15) were maintained on bromperidol therapy given in tablet form[6] once daily. Whole blood samples were collected by venipuncture using heparinized evacuated blood collection tubes (10–15 mL) prior to medication over a period of several days (n = 4–10, Table II). Each whole blood sample was centrifuged (~500×g for 10 min at 4°C), and the plasma was separated and frozen. At an appropriate time the plasma samples were thawed, and aliquots (1 mL) were transferred to test tubes and sealed with a polytef screw cap. The aliquots were refrozen and maintained at ~ −20°C until analysis could be performed (usually within 4 weeks) by the RIA procedure. Duplicate 1-mL aliquots of selected plasma samples were assayed in parallel to estimate between-sample variability.

RESULTS AND DISCUSSION

A history of standard curve parameters for the bromperidol RIA was compiled. The mean (±SD) B_0 value[7], slope, intercept, D_{50}, and minimal detectable amount, as described by the data handling computer program (6) calculated from 10 assays, were 39.8% (5.1%), −1.06 (0.06), −1.13 (0.19), 0.35 ng (0.06 ng), and 0.03 ng (0.01 ng), respectively. The mean extraction efficiency for the quality control plasma samples (n = 227) assayed in the 10 assays was 84.6 ± 7.3%. This sample extraction step was necessary to reduce the variability observed when the plasma sample was assayed directly and to separate bromperidol from its polar metabolites. A similar extraction procedure was used by Van den Eeckhout *et al.* (2) to eliminate interfering plasma constituents.

[6] In house, bromperidol, Cilag-Chemie, Schaffhausen, Switzerland.

[7] B_0 is the amount of radioactivity bound to the antisera when no unlabeled drug is present.

Table II—Patient Plasma Bromperidol Levels

Patient	Dose mg/kg	Daily Dose[a], mg	Mean ± SD[b], ng/mL	CV[c], %	N[d]
A	0.15	10	3.73 ± 0.94	25.1	9
B	0.10	10	1.65 ± 0.97	58.5	5
C	0.14	12	6.67 ± 1.31	19.6	10
D	0.23	18	8.25 ± 1.83	22.2	9
E	0.35	20	12.02 ± 5.06	42.1	4
F	0.33	20	7.62 ± 3.13	41.0	4
G	0.26	20	7.14 ± 1.19	16.7	10
H	0.51	30	10.15 ± 4.48	44.1	9
I	0.44	30	10.36 ± 1.44	13.9	10
J	0.44	30	12.77 ± 5.19	42.7	7
K	0.55	50	9.20 ± 0.42	4.6	4
L	0.93	50	10.72 ± 0.94	8.8	10
M	0.55	50	20.24 ± 2.11	10.4	7
N	0.91	60	55.13 ± 14.85	26.9	9
O	1.00	60	32.23 ± 4.12	12.8	9

[a] Administered once a day in tablet form. [b] Determined from plasma samples collected prior to dose administration (C_{min}). [c] Determined as follows: 100 × (SD/mean). [d] Number of days C_{min} blood samples were collected.

The RIA procedure for the measurement of bromperidol levels in human plasma was demonstrated by investigating the following criteria: (*a*) accuracy—correlating measured bromperidol in human plasma samples (quality control plasma samples) containing known amounts of drug (*i.e.*, 1, 5, 10, 25, 50, 100, and 150 ng/mL of bromperidol; (*b*) precision—determining replicate, within-assay, and between-assay variability statistics; and (*c*) selectivity—determining cross-reactivity characteristics of several structurally related compounds and potential metabolites of bromperidol. Table III summarizes the results obtained from the analysis of approximately five samples of each quality control human plasma specimen in several different assays performed over a 6-month period. (Note that not all quality control plasma concentrations were analyzed in every assay.) The mean values of the observed bromperidol content and between-assay variation, as well as the within-assay and replicate variations for each of the quality control plasma samples, are shown.

The quality control plasma sample concentrations of 1–50 ng/mL (reported in Table III) were compared with the expected (theoretical) values. Linear regression of the observed *versus* expected data yielded $y = 1.026x + 1.317$, with a correlation coefficient of 0.9994. If the 100- and 150-ng/mL quality control plasma results were included in this correlation, linear regression yielded $y = 1.051x + 0.889$, with a correlation coefficient of 0.9999. (Note that there were a limited number of assays, n = 3 and 2, which included the 100- and 150-ng/mL samples, respectively.)

The mean coefficient of variation [CV = 100 × (SD/mean)] observed between sample replicates (triplicate) for the seven quality control plasma concentrations ranged from 6 to 9.6%. Likewise, the mean CV observed between samples within an assay ranged from 5.4 to 12.4%, and the CV between assays ranged from 2.1 to 21.4% for the seven quality control plasma concentrations. In both instances, the within- and between-assay precision was greatest at the higher bromperidol concentrations and lowest at the 1-ng/mL concentration. These results demonstrate the stability of bromperidol in the biological matrix under the storage conditions and reasonable reproducibility of the RIA throughout the study.

The selectivity of the RIA for bromperidol is summarized in Table I, which lists the compounds examined for cross-reactivity characteristics with the haloperidol antisera. As can be seen, bromperidol (I) and haloperidol (II) show almost identical cross-reactivity characteristics with the antisera. Of the remaining compounds examined, only dehydrobromperidol (IV) showed appreciable cross-reactivity with the antisera (~25%). However, interference of dehydrobromperidol with the analysis of bromperidol in plasma is not likely to occur since there is no suggestion that this compound is a metabolite of bromperidol[8].

[8] Bromperidol is extensively metabolized in humans with the major metabolite being an *O*-glucuronide conjugate of the intact drug. Oxidative-*N*-dealkylation does not appear to be a major metabolic pathway of the drug in humans. These results have been presented at the 23rd Annual American Society of Pharmacognosy Meeting in Pittsburgh, Pa., August 1982.

Table III—Radioimmunoassay Statistics for Bromperidol Determination in Human Plasma Quality Control Samples

	Bromperidol Concentration ng/ml						
	1[a]	5[a]	10[a]	25[b]	50[b]	100[c]	150[c]
Number of assays	7	10	10	10	10	3	2
Total number of samples	22	46	46	46	46	13	8
Bromperidol concentration							
Mean ± *SD*	1.49 ± 0.32	6.38 ± 0.99	12.60 ± 1.52	27.14 ± 3.80	52.37 ± 3.89	105.35 ± 4.51	159.14 ± 3.27
n	7	10	10	10	10	3	2
Between-assay *CV*[d], %	21.2	15.6	12.1	14.0	7.4	4.3	2.1
Within-assay sample variation *CV*[e], %							
Mean ± *SD*	12.2 ± 9.4	10.3 ± 4.4	7.1 ± 4.1	7.7 ± 3.8	8.91 ± 3.8	8.2 ± 4.3	5.4 ± 0.5
n	7	10	10	10	10	3	2
Sample replicate variation *CV*[f], %							
Mean ± *SD*	9.6 ± 5.9	6.0 ± 3.8	6.0 ± 3.4	7.1 ± 4.5	8.8 ± 4.4	6.0 ± 5.1	8.1 ± 6.3
n	22	46	46	46	46	13	8

[a] Quality control plasma samples were reconstituted to original sample volume (1 mL) with RIA buffer and assayed directly by RIA method. [b] Quality control plasma samples were diluted 1:2 after reconstitution with RIA buffer prior to being assayed by RIA method. [c] Quality control plasma samples were diluted either 1:2, 1:3, or 1:9 after reconstitution with RIA buffer prior to being assayed by RIA method. [d] The mean (±*SD*) and CV of the bromperidol concentration was calculated averaging the mean bromperidol concentration of each assay for each quality control plasma concentration. [e] The mean ± *SD* of the CV observed between samples within an assay was calculated averaging the mean CV between samples of each assay for each quality control plasma concentration. [f] The mean ± *SD* of the CV of the replicates of all samples assayed for each quality control plasma concentration.

Bromperidol levels (C_{min}) determined in the patient plasma samples are summarized in Table II. These data represent the mean bromperidol concentrations determined in samples collected immediately before the daily medication (n = 4–10 d) was administered. The patient plasma samples generally demonstrated the same degree of precision described for the quality control plasma samples with regard to replicate and within-assay variability. These variabilities (*CV*) were 7.1 ± 3.9 (n = 101) and 6.6 ± 4.6 (n = 45), respectively. However, intrasubject variability, the variability observed between the C_{min} values of an individual, ranged from 4.6 to 58.5% with no apparent correlation between the *CV* and the level of drug measured (Table II). In addition, comparison of the mean C_{min} drug levels from subjects receiving approximately the same dose per body weight demonstrated substantial intersubject variability. Specifically, patients A and C (dose, 0.14–0.15 mg/kg) had mean C_{min} levels of 3.73 and 6.67 ng/mL, respectively; patients K and M (dose, 0.55 mg/kg) had mean C_{min} levels of 9.20 and 20.24 ng/mL, respectively; and patients L, N, and O (dose, 0.91–1.00 mg/kg) had mean C_{min} levels of 10.72, 55.13, and 32.23, respectively. Realistically, this intra- and interindividual variability observed in mean C_{min} bromperidol levels cannot be explained solely by assay variability. Rather a combination of metabolism, elimination, and possibly concurrent medication may significantly influence the scatter in these single time point determinations.

In conclusion, this report describes an RIA procedure used to assay the bromperidol content of human plasma samples. Predose plasma samples, obtained from schizophrenic patients chronically receiving bromperidol therapy, were assayed using this RIA method. Substantial intra- and intersubject variabilities in the C_{min} drug levels were demonstrated. The bioavailability and pharmacokinetic information of bromperidol in schizophrenic patients will be reported elsewhere.

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Novel Concept for a Mucosal Adhesive Ointment

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Received December 14, 1982, from the *Institut für Pharmazeutische Technologie der Philipps Universität Marburg/Lahn, Ketzerbach 63, 3550 Marburg*, and the [‡]*Hautabteilung des Luisen-Hospitals, Aachen, West Germany.* Accepted for publication March 8, 1983.

Abstract □ Conventional adhesive ointments cause irritation to the mucous membranes. Therefore, a novel mucosal adhesive ointment based partly on neutralized polymethacrylic acid methyl ester was formulated. The flow curves of the ointment vehicle showed pseudoplastic properties. The rheological behavior as well as the adhesion on the mucosal membrane could be varied by the type and concentration of the polymer used and the base used for neutralization. During clinical studies, the ointment vehicle as well as a tretinoin (vitamin A acid) preparation for the treatment of lichen planus did not cause any local irritation or systemic side effects. Both vehicle and preparation were found to be pleasant for the patients to use. The new system of the mucosal adhesive ointment is not limited to the incorporation of tretinoin as the active agent; combined with other drugs the system could be applied to all types of mucosal membranes.

Keyphrases □ Adhesive ointment, mucosal—tretinoin, irritation-free formulation, clinical assessment □ Lichen ruber planus—treatment with tretinoin, irritation-free formulation, mucosal adhesive ointment □ Tretinoin—mucosal adhesive ointment, irritation-free formulation, clinical testing for lichen ruber planus

Ointments employed for the treatment of the mucous membranes of the mouth must be suited to the special conditions pertaining to that particular site of application. The flow of saliva and the mechanical stress generated by the continuous movements of the buccal cavity prevent any long-term adhesion of the ointment subsequent to its application. The quantity

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