Direct Radioimmunoassay for Haloperidol in Human Serum

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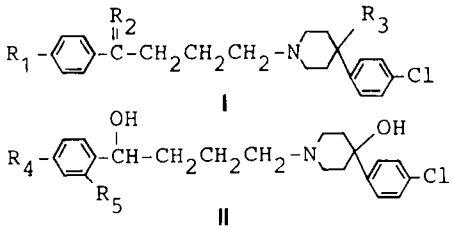
Abstract □ A direct radioimmunoassay for the accurate determination of haloperidol in human serum has been developed. Based on recent information about the metabolism of haloperidol, a new haloperidol hapten, in which a (3-carboxypropionyl)methylamino group was attached as a bridge in the place of fluorine atom, was synthesized and coupled to bovine serum albumin through the bridge to provide a new immunogen. Guinea pigs were used for the immunization. Since the antisera obtained by the new immunogen still cross reacted >10% with reduced haloperidol, the immunological tolerance to reduced haloperidol was induced by administration of a copolymer of D-glutamic acid and D-lysine linked with reduced haloperidol. This gave an antiserum in guinea pigs which was highly specific for unchanged haloperidol with negligible cross reactivity (≦1.0%) to any haloperidol metabolites including the newly found ones. With the newly developed antiserum and [^{3}H]haloperidol, serum haloperidol levels can be determined over the concentration range from 0.3 to 20 ng/mL, using 0.1 mL of human serum, without an extraction procedure.

Haloperidol (Table I) is a butyrophenone neuroleptic drug that is widely used in many countries. Since it was pointed out that monitoring the blood levels of the unchanged drug was helpful for the rational clinical use of the drug, several methods for the determination of haloperidol in serum or plasma have been developed. These include gas chromatography (GC), radioimmunoassay (RIA), high-performance liquid chromatography, etc.[1–5] Among these methods, RIA is extremely desirable because of its high sensitivity and ease with which routine analysis can be performed for therapeutic monitoring of this drug. Several investigators reported the RIAs of haloperidol in which the antisera were raised in animals immunized with the immunogens shown in Table I [(f)–(h)].[2,4,5] However, the antisera obtained by the use of immunogen A or B (antisera A or B) cannot avoid the cross reaction with reduced haloperidol [Table I (b)]. Therefore, the accurate determination of haloperidol in human serum by the reported RIAs based on antisera A or B was not possible because of interference from the presence of reduced haloperidol in humans receiving haloperidol.[6,7] On the other hand, antisera C did not cross react with reduced haloperidol. The overestimation nevertheless occurred when human serum samples were measured by the RIA with antisera C.[6] Van den Eeckhout et al.[8] pointed out that one or more unknown polar metabolites which cross reacted with antisera C were present in human serum. Recently, Oida et al.[9] isolated and identified the three polar metabolites of haloperidol from human urine; that is, sulfuric acid (MH-1) and glucuronic acid (MH-2) conjugates of the reduced and oxidized metabolites of haloperidol, and *O*-glucuronide of haloperidol (MH-3) shown in Table I [(c)–(e)]. One of these metabolites, MH-3, did cross react with antisera C, and was found in patient plasma about twice as much as unchanged haloperidol after administration of haloperidol decanoate [MH-3:haloperidol ratio: 2.03 ± 1.07 (mean ± SD; n = 11)].[10] Therefore, MH-3 is considered to be responsible for the overestimation of the RIA with antisera C.

In order to develop a convenient direct RIA, we tried to obtain a specific antiserum which did not cross react with either reduced haloperidol or MH-3. We synthesized the new immunogen [Table I (i)], and utilized the devised immunization method reported by Tateishi et al.[11] in which the immunological tolerance to a structurally related metabolite was induced by the copolymer of D-glutamic acid and D-lysine (D-GL). The obtained antiserum made it possible to develop the direct RIA of haloperidol in human serum. In this paper, the details about the development of the direct RIA are described.

Table I—Structures of Haloperidol, Reduced Haloperidol, Polar Metabolites, Immunogens, and D-GL Conjugate



I

II

	Name	Structure	Substituent Group
(a)	Haloperidol	I	R_1:F, R_2:O, R_3:OH
(b)	Reduced haloperidol	II	R_4:F, R_5:H
(c)	MH-1	II	R_4:F, R_5:O-SO_3H
(d)	MH-2	II	R_4:F, R_5:O-$C_6H_9O_6$
(e)	MH-3	I	R_1:F, R_2:O, R_3:O-$C_6H_9O_6$
(f)	Immunogen A	I	R_1:F, R_2:N-NHCO-BSA, R_3:OH
(g)	Immunogen B	I	R_1:F, R_2:N-OCH_2CONH-BSA, R_3:OH
(h)	Immunogen C	I	R_1:F, R_2:O, R_3:O-$COCH_2CH_2CONH$-BSA
(i)	New immunogen	I	R_1:N(CH_3)$COCH_2CH_2CONH$-BSA, R_2:O, R_3:OH
(j)	D-GL conjugate	II	R_4:N(CH_3)$COCH_2CH_2CONH$-D-GL, R_5:H

Experimental Section

Chemicals—[^{3}H]Haloperidol with a specific activity of 20 Ci/mmol was purchased from I. R. E. (Fleurus, Belgium) and the radiochemical purity was checked by thin-layer chromatography prior to use. Haloperidol and biperiden were obtained from Dainippon Pharmaceutical Company (Osaka, Japan). Floropipamide, pimozide, chlorpromazine, trihexyphenidyl, and promethazine were extracted with solvent from their commercial preparations and were purified. 4-(*p*-Chlorophenyl)-4-hydroxypiperidine and spiroperidol were kindly supplied by Janssen Pharmaceutica (Beerse, Belgium). Reduced haloperidol and 4-fluorophenylaceturic acid were synthesized by the methods of Oida et al.[9] and Braun et al.,[12] respectively. The metabolites MH-1, MH-2, and MH-3 were prepared according to the method of Oida et al.[9] from patient urine. 4-Fluorobenzoylpropionic acid, 4-fluorophenylacetic acid, and amantadine were purchased from Aldrich Chemical Company (Milwaukee, WI), bovine serum albumin (BSA) from Sigma Chemical Company (St. Louis, MO), human serum from Flow Laboratories (Rockville, MD), and Freund's complete

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adjuvant from Difco Laboratories. The random copolymer of D-glutamic acid and D-lysine (D-GL) was purchased from Miles Laboratories, Inc. (Elkhart, IN). The copolymer had an average molecular weight of 47 500 and a G:L ratio of 60:40. All other chemicals used were of analytical reagent grade.

Preparation of Immunogen—Immunogen was prepared as shown in Scheme I.

Synthesis of 4'-(Cyclopropylcarbonyl)-N-methylacetanilide (2)—A solution of 4'-(4-chlorobutyryl)acetanilide[13] (**1**; 40 g) in 200 mL of absolute dimethylformamide (DMF) was added in a dropwise manner to a suspension of 20 g of NaH (in oil, content ~50%) in 280 mL of DMF. After the mixture was stirred for 1 h, 31.2 mL of CH_3I was added in a dropwise manner to the mixture at 20–30 °C. The resultant mixture was subsequently stirred for 2 h and evaporated under reduced pressure to remove DMF. Ice water (500 mL) was added to the residue, and the resultant solution was extracted twice with 500 mL of $CHCl_3$. The organic layer was dried over anhydrous Na_2SO_4 and evaporated under reduced pressure. The residue was purified by column chromatography on silica gel (440 g). The fractions eluted with $CHCl_3$ were collected, evaporated, and recrystallized from ethyl acetate-*n*-hexane to give 15.96 g of **2**, mp 80–82 °C; MS: (*m/z*) 217 (M^+); IR (KBr): ν_{max} 1655 (C=O) cm^{-1}; ^1H NMR ($CDCl_3$): δ 0.80–1.50 (4H, m, CH_2CH_2), 1.97 (3H, s, $COCH_3$), 2.69 (1H, m, CH), 3.33 (3H, s, NCH_3), 7.33, and 8.10 ppm (4H, AA'BB' type aromatic H).

Anal.—Calc. for $C_{13}H_{15}NO_2$: C, 71.87; H, 6.96; N, 6.45. Found: C, 71.71; H, 7.04; N, 6.47.

Synthesis of 4-Chloro-4'-(N-methylacetamido)butyrophenone (3)—A mixed solution of 30% HCl in isopropanol (150 mL) and water (16 mL) was added to 13.5 g of **2**. This was stirred for 7 h at room temperature; the resultant mixture was left overnight and evaporated under reduced pressure. Water was added to the residue, and the resultant solution was made alkaline with saturated Na_2CO_3 solution and extracted twice with 500 mL of ethyl acetate. The organic layer was dried over anhydrous Na_2SO_4, evaporated, and dried under reduced pressure to give 15.8 g of **3** as a colorless oil.

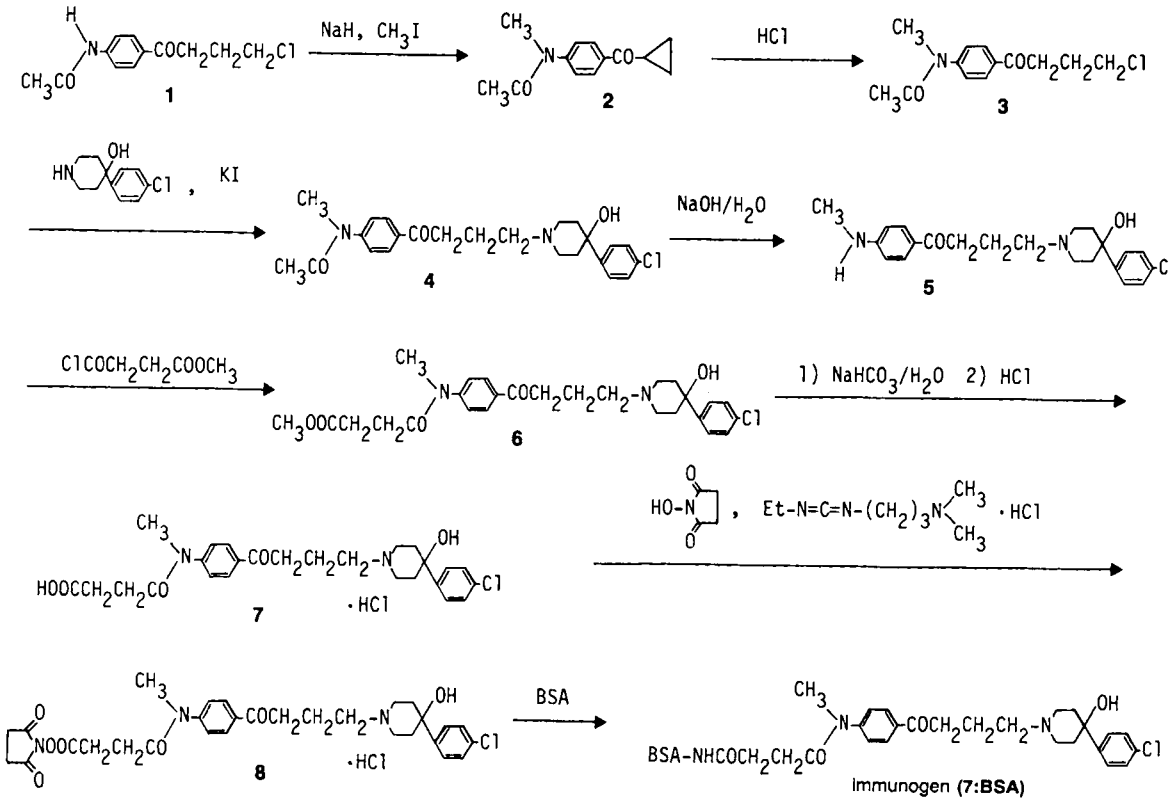
Synthesis of 4-[4-(p-Chlorophenyl)-4-hydroxypiperidino]-4'-(N-methylacetamido)butyrophenone (4)—Compound **3** (15.8 g) and 4-(*p*-chlorophenyl)-4-hydroxypiperidine (27.6 g) were dissolved in isobutyl methyl ketone, and KI (1.1 g) was added to the solution. The mixture was refluxed for 7 h, cooled in an ice bath, and filtered. The filtrate was evaporated, and the residue was purified by column chromatography on silica gel (250 g). The fractions eluted with acetone:toluene (1:2) and acetone were collected, evaporated, and recrystallized from ethyl acetate-*n*-hexane to give 16.42 g of **4**, mp 121–123 °C.

Synthesis of 4-[4-(p-Chlorophenyl)-4-hydroxypiperidino]-4'-(N-methylamino)butyrophenone (5)—A solution of **4** (16.42 g) in 1962 mL of ethanol was mixed with 197 mL of a 10% NaOH aqueous solution. The mixture was refluxed for 15 h and concentrated to ~100 mL under reduced pressure. The resultant mixture was diluted with 100 mL of water and concentrated again to 100 mL; this procedure was repeated three times. The concentrated mixture was cooled in an ice bath and filtered. The precipitate was washed with 300 mL of cold water and recrystallized from isopropanol to give 13.34 g of **5**, mp 155–157 °C; MS: (*m/z*) 386 (M^+); IR (KBr): ν_{max} 1655 (C=O) cm^{-1}; ^1H NMR ($CDCl_3$): δ 1.50–3.10 (15H, $COCH_2CH_2CH_2$, piperidine H, OH), 2.91 (3H, d, J = 7 Hz, NCH_3), 4.20 (1H, q, J = 7 Hz, NH), 6.55, 7.85 (4H, AA'BB' type, aromatic H), and 7.15–7.55 ppm (4H, m, chlorobenzene H).

Anal.—Calc. for $C_{22}H_{27}N_2O_2Cl$: C, 68.29; H, 7.03; N, 7.24; Cl, 9.16. Found: C, 68.10; H, 6.97; N, 6.96; Cl, 9.16.

Synthesis of 4-[4-(p-Chlorophenyl)-4-hydroxypiperidino]-4'-[N-(3-methoxycarbopropionyl)methylamino]butyrophenone (6)—A solution of β-carbomethoxypropionyl chloride[14] (5.19 g) in 200 mL of absolute ether was added in a dropwise manner to 13.34 g of **5** dissolved in 133 mL of pyridine with stirring. The reaction mixture was subsequently stirred for 2.5 h and evaporated under reduced pressure. The residue was dissolved in 100 mL of water, made alkaline with a saturated K_2CO_3 solution, and extracted with 500 mL of $CHCl_3$. The organic layer was dried over anhydrous Na_2SO_4 and evaporated. The residue was purified by column chromatography on silica gel (300 g). The fractions eluted with $CHCl_3$:CH_3OH (4:1) were collected, evaporated, and recrystallized from toluene-*n*-hexane-ether to give 8.34 g of **6**; mp 114–116 °C.

Synthesis of 4-[4-(p-Chlorophenyl)-4-hydroxypiperidino]-4'-[N-(3-carboxypropionyl)methylamino]butyrophenone Hydrochloride (7)—A solution of **6** (4.0 g) in 448 mL of ethanol was mixed with 224 mL of a 1.8% $NaHCO_3$ aqueous solution. The mixed solution was refluxed for 1.5 h and evaporated under reduced pressure. The residue was dissolved in 30 mL of water, neutralized with acetic acid, and decanted. The residual oil was purified by column chromatography on silica gel (73 g). The fractions eluted with acetone and $CHCl_3$-CH_3OH



Scheme I—Synthesis of new immunogen (**7**:BSA).

(3:2) were collected and evaporated. The residue was dissolved in 33 mL of dioxane, made acid with conc. HCl dissolved in dioxane, and evaporated under reduced pressure. The residue was first recrystallized from CH_3OH-ether and further recrystallized from ethanol to give 1.57 g of **7**; mp 180–182 °C. MS: (*m/z*) 487 (M^+); IR (KBr): ν_{max} 1630, 1650, 1690, 1710, 1730 (C=O) cm^{-1}; ^1H NMR (DMSO-d_6): δ1.70–2.55 (8H, $COCH_2CH_2CH_2$, piperidine-3,5 H), 3.10–3.55 (10H, $COCH_2CH_2CH_2$, $COCH_2CH_2CO$, piperidine-2,6 H), 3.23 (3H, s, NCH_3), 5.61 (1H, s, OH), 7.47, 7.54 (4H, AA'BB' type, chlorobenzene H), 7.55, 8.10 (4H, AA'BB' type, aromatic H), 10.77 (1H, bs, HCl), and 12.09 ppm (1H, bs, COOH).

Anal.—Calc. for $C_{26}H_{31}N_2O_5Cl \cdot HCl$: C, 59.66; H, 6.16; N, 5.35; Cl, 13.55. Found: C, 59.48; H, 6.01; N, 5.19; Cl, 13.49.

Preparation of Immunogen (7:BSA)—Compound **7** was coupled to BSA by the *N*-succinimidyl ester method. Compound **7** (700 mg) and *N*-hydroxysuccinimide (174 mg) were dissolved in 7 mL of absolute DMF, and 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (295 mg) was added to the solution with stirring. The mixture was stirred for 6 h. Then the mixture was left overnight and evaporated under reduced pressure to remove DMF. The residue was dissolved in 20 mL of water in an ice bath and extracted with 150 mL of $CHCl_3$. The organic layer was dried over anhydrous Na_2SO_4, evaporated, and recrystallized from acetone to give 641 mg of the *N*-succinimidyl ester of **7** (**8**); mp 193–195 °C (dec).

Compound **8** (100 mg) was dissolved in 5 mL of absolute DMF, and the solution was added to BSA (532 mg) dissolved in 195 mL of 0.05 M phosphate buffer (pH 7.4). This was stirred for 3 h; the mixture was dialyzed against running water for 4 d and lyophilized to give 557 mg of fluffy immunogen powder (7:BSA).

Conjugation of the haloperidol analogue to BSA was confirmed by UV spectrophotometric analysis.[15] The degree of substitution was estimated to be 11.3 mol of hapten per mol of BSA.

Preparation of Reduced Haloperidol:D-GL Conjugate—Reduced haloperidol:D-GL conjugate was prepared as shown in Scheme II.

Synthesis of 4-{4-[4-(p-Chlorophenyl)-4-hydroxypiperidino]-1-hydroxybutyl}-N-(3-methoxycarbopropionyl)-N-methylaniline (9)—First, $NaBH_4$ (0.42 g) was added to a solution of **6** (1.1 g) in 27.5 mL of methanol with stirring. The mixture was stirred for 2 h at room temperature and evaporated under reduced pressure to remove CH_3OH. The residue was dissolved in 10 mL of water and extracted twice with 100 mL of ether. The organic layer was washed with a saturated NaCl solution, dried over anhydrous Na_2SO_4, and evaporated. The residue was purified by column chromatography on silica gel (23.8 g). The fractions eluted with acetone-*n*-hexane (1:9) and acetone-*n*-hexane (1:1) were collected and evaporated to give 1.02 g of **9** as a colorless oil.

Synthesis of 4-{4-[4-(p-Chlorophenyl)-4-hydroxypiperidino]-1-hydroxybutyl}-N-(3-carboxypropionyl)-N-methylaniline (10)—Compound **9** (518 mg) was mixed with 51.6 mL of diluted ethanol (17%), and the mixture was refluxed for 14 h and evaporated under reduced pressure. The residue was dissolved in 20 mL of water in an ice bath. The resultant solution was washed twice with 50 mL of ether and evaporated under reduced pressure, keeping the temperature below 45 °C, to give 382 mg of **10**, mp 117–120 °C; MS: (*m/z*) 489 (MH^+); IR(KBr): ν_{max} 1640 (C=O) cm^{-1}; ^1H NMR (DMSO-d_6): δ 1.40–2.80 (18H, $COCH_2CH_2CH_2$, $COCH_2CH_2CO$, piperidine H), 3.16 (3H, s, NCH_3), 4.60 (1H, t, J = 6.2 Hz, CHOH), 7.30, 7.43 (4H, AA'BB' type, aromatic H), 7.39, and 7.52 ppm (4H, AA'BB' type, chlorobenzene H).

Anal.—Calc. for $C_{26}H_{33}N_2O_5Cl \cdot 2H_2O$: C, 59.48; H, 7.10; N, 5.34; Cl, 6.57. Found: C, 59.21; H, 7.29; N, 5.41; Cl, 6.63.

Preparation of Reduced Haloperidol:D-GL Conjugate (10:D-GL)—To a solution of **10** (50 mg) in 2.5 mL of *N*, *N*-dimethylformamide was added 40 μL of triethylamine. The mixture was stirred for 30 min at 20 °C and cooled to 5–10 °C. Isopropyl chloroformate (17.6 mg) was added to the mixture, and the mixture was stirred for 20 min at 5–10 °C and subsequently for 2 h at 20 °C. The mixture was added in a dropwise manner to a solution of D-GL (160 mg) in 12 mL of 0.05 M phosphate buffer (pH 7.8). The resultant mixture was stirred for 3 h at 20 °C and centrifuged at 1000 × *g* for 15 min. The supernatant was dialyzed against distilled water for 3 d at 4 °C and lyophilized to give 83.5 mg of fluffy powder (**10**:D-GL).

Conjugation of the reduced haloperidol analogue to D-GL was confirmed by the analysis of ε-amino group remaining in the conjugate by utilizing ninhydrin reaction.[16] The degree of substitution was estimated to be ~9 mol of hapten per mole of D-GL.

Immunization Procedures—Three groups of guinea pigs with 2–3 animals per group were given a primary immunization intramuscularly (im) with 1 mg of 7:BSA emulsified in Freund's complete adjuvant (FCA). After 3 weeks, all guinea pigs received a secondary challenge im with 1 mg of 7:BSA in FCA. They were then boosted with 1 mg of 7:BSA in FCA three times at 2-week intervals. Group 2 was pretreated with a single intraperitoneal injection of 10 mg of **10**:D-GL in saline 3 d before the primary injection of 7:BSA. Group 3 received two injections of 10 mg of **10**:D-GL, one on day 18, 3 d before the secondary stimulation with 7:BSA, and one on day 32, 3 d before the tertiary stimulation with 7:BSA. Group 1 received only saline instead of **10**:D-GL. Blood was collected by cardiac puncture 12 d after the final booster injection, and the serum was separated and stored at −20 °C.

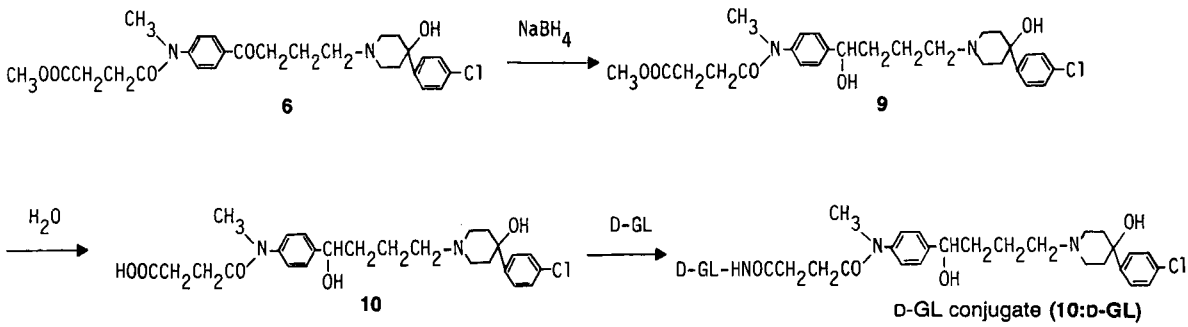
Assay Procedure—The following solutions were prepared prior to assay: 0.075 M phosphate buffer, pH 7.4 (buffer A); 0.075 M phosphate buffer containing 0.2% BSA (buffer B); standard solutions containing 0.03, 0.06, 0.125, 0.25, 0.5, 1, and 2 ng of unlabeled haloperidol in 0.1 mL of buffer B; a solution of [^3H]haloperidol in buffer B to give 2.2 × 10^4 dpm/188 pg/0.1 mL; and dextran-coated charcoal (DCC) suspension containing 500 mg of activated charcoal and 50 mg of dextran T-70 in 100 mL of buffer A.

One tenth milliliter of serum samples or control serum, 0.1 mL of buffer B or standard solutions, 0.7 mL of buffer A, 0.1 mL of [^3H]haloperidol, and 0.1 mL of diluted antiserum (diluted 1:8000 with buffer B) were added to each of the plastic tubes. The contents of the tubes were mixed on a vortex mixer and allowed to stand for 1 h at room temperature (20 ± 5 °C). After the mixture had been cooled to 4 °C, 0.2 mL of DCC suspension was added to the mixture. The tubes were mixed by vortex vigorously, allowed to stand for 1 h at 4 °C, and centrifuged at 1000 × *g* for 15 min. One milliliter of the supernatant was pipetted into a counting vial containing 10 mL of scintillation cocktail (Aquasol 2, New England Nuclear Corporation, Boston, MA) and the radioactivity was counted in a scintillation spectrometer (Packard Tri-Carb, model 2450).

A standard curve was constructed by a logit–log plot of the relative percentage (B/B_O) of bound labeled drug against the amount of unlabeled haloperidol added.

Antibody specificity was evaluated by measuring the inhibition of the antibody:haloperidol binding caused by increasing amounts of each of the related compounds which included the metabolites of haloperidol, structurally related and unrelated neuroleptic drugs, as well as antiparkinsonian drugs.

Gas Chromatography—To 2 mL of plasma in a centrifuge tube



Scheme II—Synthesis of D-GL conjugate (**10**:D-GL).

were added 2 mL of 1 M NaOH, 0.05 mL of internal standard solution (1-[3-(4-fluorobenzoyl)propyl]-4-(4-methoxybenzyl)piperazine dihydrochloride, 60 ng/0.05 mL), 0.05 mL of acetone or standard solution of haloperidol, and 9 mL of *n*-heptane containing 1.5% isoamyl alcohol. The tube was shaken for 10 min and centrifuged. The organic layer (8 mL) was transferred to another centrifuge tube and extracted into 5 mL of 0.05 M H_2SO_4. The aqueous layer (4 mL) was separated and made alkaline with 1 mL of 1 M NaOH, and then extracted with 0.1 mL of toluene. The organic layer (0.02 mL) was injected into the gas chromatograph.

A Hewlett-Packard model 5840A equipped with NPD was used. A silanized glass column (100 cm × 2 mm i.d.) was packed with 3% OV-17 on HP Chromosorb W (100–120 mesh). Helium was used as the carrier gas at a flow rate of 40 mL/min. The column temperature was 250 °C and the injector and detector temperatures were 300 °C.

Results

Antibody Titer—The titer of each antiserum was examined according to the procedure described above. All antisera obtained had high titers. The dilutions of the antisera which gave 50% binding were 1:88 000 to 1:198 000 in final dilution.

Cross Reactivity with Reduced Haloperidol—The cross reactivity of each antiserum with reduced haloperidol was examined. As shown in Table II, the cross reactivities of antisera obtained from groups II and III were remarkably lower than those of group I. For example, the antiserum III-2 gave ~1% cross reactivity with reduced haloperidol. Therefore, the antiserum III-2 was chosen for the direct RIA of haloperidol in human serum. The following investigation was performed with the antiserum III-2.

Sensitivity of the Assay—A typical standard curve obtained with 1:100 000 antiserum dilution and 150 pg of ^{3}H-labeled haloperidol was the straight line with the following equation when plotted on a logit-log scale:

$$\text{logit}(B/B_0) = -0.801\ 1n\ X + 2.682 \qquad (1)$$

where X is the amount of haloperidol added. Haloperidol can be reliably assayed in the range of 30–2000 pg/0.1 mL of serum.

Precision of the Assay—Intra- and interassay variations were assessed by four times repeated assays of pooled human sera (concentration range ~1–20 ng/mL). The coefficients of variation ranged from 2.7 (12.1 ng/mL) to 9.8% (1.1 ng/mL) for intra-assay, and from 5.0 (4.2 ng/mL) to 12.3% (1.1 ng/mL) for interassay.

Specificity of the Antiserum III-2—The cross reactivities of various compounds with the antiserum III-2 are shown in Table III. The antiserum was highly specific for unchanged haloperidol with negligible cross reactivity to any haloperidol metabolites including the newly found ones. No cross reaction was observed with the antiparkinsonian drugs tested which are generally administered with haloperidol.

Table II—Cross Reactivity of Antihaloperidol Antisera with Reduced Haloperidol

Group	Animal No.	% Cross Reactivity (B/B_0)[a]		
		20%	50%	80%
I	1	23.7	37.2	58.2
	2	10.6	18.0	30.7
	3	85.3	101.5	120.3
II	1	1.0	2.3	5.7
	2	1.5	2.6	4.6
	3	2.0	3.5	6.2
III	1	1.0	1.5	2.2
	2	0.7	1.0	1.3

[a] The percent cross reactivity was calculated at 20, 50, and 80% displacement of radioligand.

Table III—Specificity of Antiserum III-2

Compound	% Cross Reactivity[a]
Haloperidol	100
Haloperidol metabolites	
4-Fluorophenylaceturic acid	<0.1
4-Fluorobenzoylpropionic acid	<0.1
4-Fluorophenylacetic acid	<0.1
4-(*p*-Chlorophenyl)-4-hydroxypiperidine	<0.1
Reduced haloperidol	1.0
MH-1	0.7
MH-2	0.6
MH-3	<0.1
Antipsychotic drugs	
Floropipamide	<0.1
Spiroperidol	<0.1
Pimozide	<0.1
Chlorpromazine	<0.1
Antiparkinsonian drugs	
Biperiden	<0.1
Amantadine	<0.1
Trihexyphenidyl	<0.1
Promethazine	<0.1

[a] The percent cross reactivity was calculated at 50% displacement of radioligand.

Comparison of Radioimmunoassay and Gas Chromatography—Twenty-eight human serum samples of haloperidol were determined by both RIA and GC. The results are shown in Figure 1. There is a good correlation between values obtained by the two methods (correlation coefficient, 0.990). The slope value (a) and intercept (b) of the regression line are 0.971 ± 0.055 and 0.313 ± 2.586 (95% confidence interval), respectively, when applied to the equation $Y = aX + b$, where X and Y are values obtained by RIA and GC. The intercept is not significantly different from zero, and the confidence limit ($p = 0.05$) of this slope encompasses the ideal slope of unity.

Discussion

In the development of RIAs for drugs, the cross reactivity of the employed antisera with the metabolites of the drug and

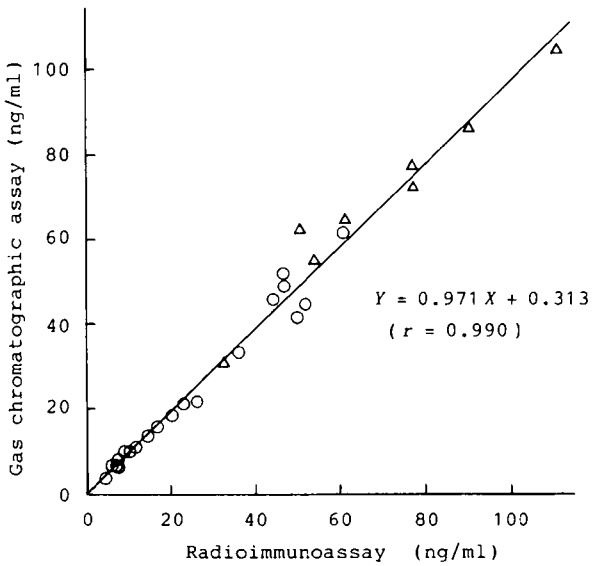


Figure 1—Comparison of radioimmunoassay and gas chromatographic assay of haloperidol in serum. Serum samples were obtained from patients after oral (○) or intravenous (△) administration of haloperidol. The line was drawn from the values calculated by the method of least squares.

other coadministered agents is the most important determinant of the specificity of the assay procedure. Designing immunogens for the preparation of specific antisera requires adequate knowledge about the metabolism of drugs. Since haloperidol had been believed to be metabolized only by the oxidative *N*-dealkylation, the development of direct RIAs was attempted by the use of the antisera obtained by the immunogens shown in Table I [(f)–(h)] until the beginning of the 1980s.[2,4,5] However, Rubin et al.[6] pointed out that in human serum the values determined by these RIAs were higher than those determined by GC. Before this publication, Forsman et al.[7] reported that reduced haloperidol was present as a metabolite in human serum. On account of these findings, the conventional RIAs were re-evaluated and it has been reported that a prior extraction step is necessary for the accurate determination of haloperidol in human serum.[17]

We investigated the metabolism of haloperidol in humans and it became clear to us that the antiserum which does not cross react with either reduced haloperidol or MH-3 was required for the development of a direct RIA. In order to obtain such a specific antiserum, we synthesized the immunogen whose coupling site to BSA was different from either the hydroxy group or carbonyl group of haloperidol [Table I (i)]. However, the antisera obtained in guinea pigs by the new immunogen cross reacted >10% with reduced haloperidol, with no cross reactivity to MH-3. Korpi et al.[18] have reported the presence of 20–200% of reduced haloperidol in human serum as compared with the unchanged drug. Therefore, the antiserum could not be used for a direct RIA. In order to overcome the problem of cross reactivity of the antiserum to reduced haloperidol, we applied a unique immunization protocol which has been developed by Tateishi et al.[11] We coupled reduced haloperidol with D-GL and injected the resultant conjugate into the animals prior to or during the immunization with the new immunogen of haloperidol. The antisera thus obtained by this immunization protocol showed much lower cross reactivities with reduced haloperidol than the antisera obtained by the conventional immunization procedures (Table II). Among the antisera developed, antiserum III-2 had the lowest cross reactivity (1%) with reduced haloperidol. Therefore, a direct RIA was developed. This RIA had negligible cross reactivity with any haloperidol metabolites such that the values obtained by this direct RIA based on antiserum III-2 were in good agreement with those determined by GC (Table III, Figure 1). The present method is the first direct RIA that enables accurate determination of haloperidol in human serum and is simple enough for use in clinical laboratories for therapeutic monitoring of haloperidol.

In the production of antisera to drugs, the coupling site of hapten to carrier protein is the most important factor which determines the specificity of the obtained antiserum. Therefore, the immunogen should be designed with a view to avoid the cross reaction with metabolites. However, sometimes this may not be possible. In such situations, an alternate strategy reported here which overcame the cross reactivity with reduced haloperidol is recommended. This strategy is based on the development of immunological tolerance which in the present situation was induced by injecting D-GL conjugate of reduced haloperidol. This resulted in obtaining an antiserum with negligible cross reactivity to reduced haloperidol.

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