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Simultaneous determination of plasma haloperidol and its metabolite reduced haloperidol by liquid chromatography with electrochemical detection
Plasma levels in schizophrenic patients treated with oral or intramuscular depot haloperidol

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Abstract

A simple and highly sensitive liquid chromatographic method with electrochemical detection for the simultaneous determination of haloperidol and its metabolite reduced haloperidol in human plasma has been developed. The sample preparation for the analysis involves a simple one-step extraction procedure with 10% methylene chloride in pentane. The compounds were separated on a cyano column maintained at a temperature of 40°C and were detected electrochemically by a flow-through analytical cell kept at +0.95 V. The standard curve is linear over the range of 0.1 to 15 ng/ml and the lower limit of quantitation is 0.1 ng/ml for haloperidol and 0.25 ng/ml for reduced haloperidol which is equivalent to approximately 40 pg on column when 1 ml of plasma was used for the analysis. The lower limit of quantitation for reduced haloperidol can be extended to 0.1 ng/ml if 2 ml of plasma is used in the analysis. The coefficient of variation of the determination of plasma levels by this method over the standard curve concentration range was less than 10%. Commonly co-administered drugs and other neuroleptics used in conjunction with haloperidol did not interfere in the determination of either haloperidol or reduced haloperidol. This method has been successfully used for the determination of haloperidol and reduced haloperidol in plasma and their levels in patients treated with various doses oral haloperidol or intramuscular haloperidol decanoate are reported.

1. Introduction

Haloperidol (HPL) is a potent butyrophenone type antipsychotic drug widely used in the treat-

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ment of schizophrenia. The metabolism of HPL [1] involves oxidative dealkylation to inactive metabolites and reduction of the keto group to hydroxyl group giving rise to reduced haloperidol (RHPL). The pharmacological activity of RHPL, a major metabolite in humans, has been variously reported as inactive or weakly active [2]. However, the clinical importance of RHPL has been suggested by reports in the literature of a higher ratio of RHPL/HPL in non-responders [3,4]. In order to understand the correlation between the plasma levels and clinical outcome, it may be important to monitor plasma levels of both HPL and RHPL in schizophrenic patients treated with either oral or i.m. HPL. Analytical methods have been reported in the literature for the determination of HPL and RHPL in biological fluids. These include radioreceptor assay [5], radioimmunoassay [6–8], GC [9–11], GC–MS [12–14] and high-performance liquid chromatography (HPLC) with UV [15–22], photodiode array [23] and electrochemical detection (ED) [24–27]. The previously reported HPLC–ED methods used 2-ml plasma samples and involved either a solid-phase extraction [26] or a two-step extraction procedure with a large excess of chlorpromazine as internal standard to increase the recovery of HPL and RHPL [27]. Due to the ready availability of HPLC systems, we developed an HPLC–ED method for the determination of plasma HPL and RHPL. The HPLC method reported here uses 1 ml of plasma and involves a simple one-step extraction procedure using chlorohaloperidol (ClHPL) as internal standard. This method has sufficient sensitivity and reproducibility for the routine plasma level determination of steady-state levels of HPL and RHPL in patients who are treated chronically with oral HPL or intramuscular HPL decanoate.

2. Experimental

2.1. Chemicals

HPL, RHPL and ClHPL were purchased from Research Biochemicals (Matick, MA, USA). All chemicals and HPLC grade solvents were procured from Fisher Scientific (Los Angeles, CA, USA). The commonly co-administered drugs and other antipsychotic drugs such as ibuprofen (Motrin), acetaminophen (Tylenol), pseudoephedrine, trihexphenidyl (Artane), benztropine (Cogentin), lorazepam (Ativan), fluphenazine (Prolixin), clozapine (Clozaril), and clonazepam (Klonapin) were obtained from Sigma (St. Louis, MO, USA). Deionized water (Ropure-Nanopure, Barnstead, MA, USA), all chemical and solvents were used without further purification. Centrifugations were carried out at 18°C in a refrigerated centrifuge (IEC, Centra 8R, American Scientific Product, Edison, NJ, USA) at 1725 *g*.

2.2. Chromatography conditions

The liquid chromatographic system consisted of a Schimadzu solvent delivery pump Model LC 600, Schimadzu autosample injector Model SIL 9A fitted with 150-μl sample loop (Cole Scientific, Moorpark, CA, USA) and an Ultrasphere cyano column (25 cm × 0.46 cm I.D., 5 μm average particle size; Beckman, San Ramon, CA, USA). The detector system consisted of a ESA Coulochem detector Model 5100A (ESA, Bedford, MA, USA) fitted with a high sensitivity analytical cell (Model 5011, ESA) and a guard cell (Model 5020, ESA). The high sensitivity analytical cell contained two flow-through low volume electrodes positioned serially. The electrodes were porous graphite in nature with large surface area. The guard cell was positioned between the solvent delivery pump and the sample injector. The electrode in the guard cell was kept at a higher potential (1 V) than the detection electrode so that the guard cell electrode reacted with most of the electrochemically active trace impurities in the mobile phase. Thus the electrodes in the analytical cell received an electrochemically clean mobile phase. The applied voltages for the electrodes 1 and 2 in the analytical cell were 0.6 and 0.95 V, respectively. Electrode 1 was the screening electrode which reacted with interfering substances, if any, present in the sample before it reached electrode 2, the detection electrode. The mobile phase consisted of an aqueous solution of 0.04 *M* am-

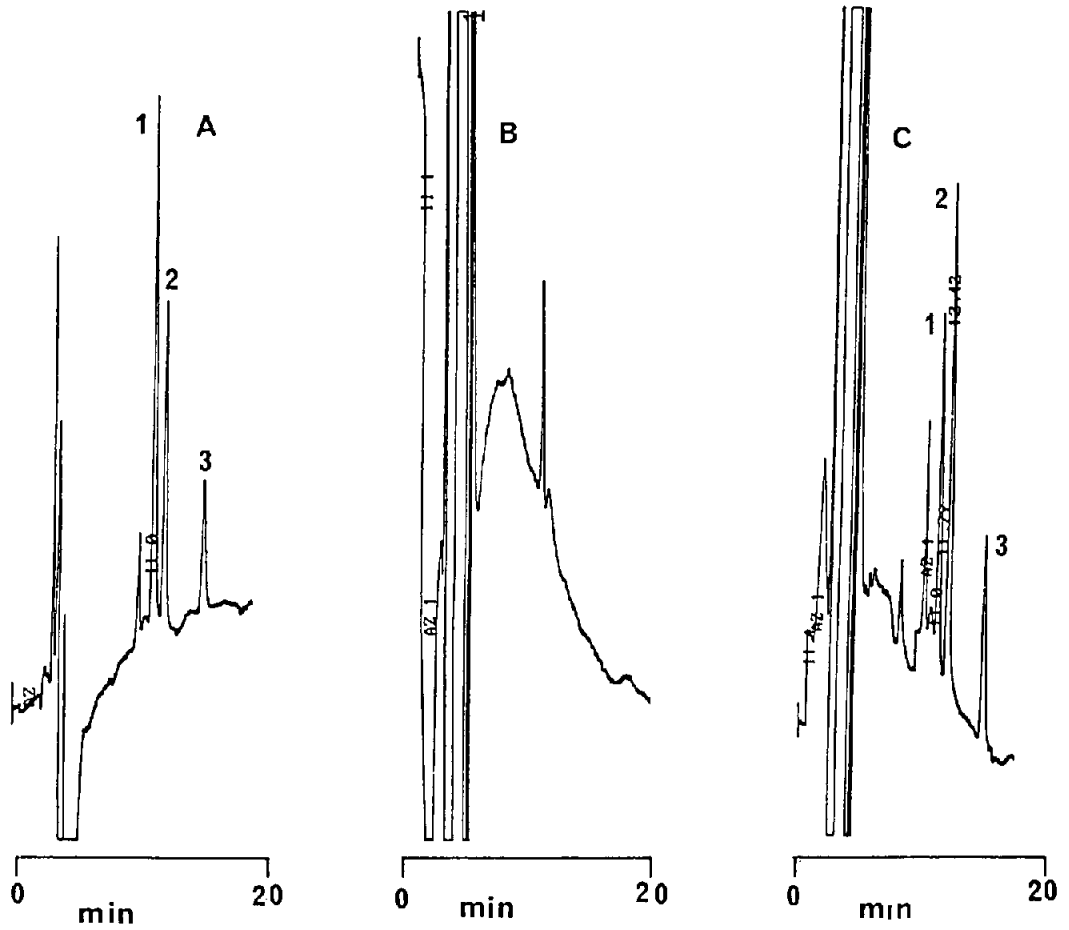

Fig. 1. Chromatograms of (A) a standard solution of (1 ng) of each of ClHPL (1), HPL (2), and RHPL (3); (B) a blank plasma extract; (C) a blank plasma spiked with 3.5 ng of each of HPL and RHPL per ml of plasma.

monium acetate (pH 6.8, not adjusted)–methanol–acetonitrile (8:6:86, v/v) and was degassed by filtering through a 0.2-μm Nylon filter before use. The cyano column was heated in a column oven (FIAtron Model TC 50, Alltech, San Jose, CA, USA) and the oven temperature was maintained at 40°C. The samples were eluted isocratically with a mobile phase flow-rate of 0.8 ml/min (60 kgf/cm^2). The detector response was recorded using a Data Jet integrator (Spectra Physics, San Jose, CA, USA).

2.3. Plasma samples

Thirteen treatment resistant schizophrenic patients treated with an oral HPL regimen were admitted to a HPL decremental dosage study where the dosage reduction schedule was 80, 65, 50, 35, 20, 15, 10, and 5 mg of HPL per day. Doses of HPL as concentrated solution were given once a day at bed time (21:00–22:00 p.m.). Venous blood samples were collected in heparinized Vacutainer tubes. The blood samples were drawn on any day in the fifth week after a scheduled dosage change in the morning (06:00–09:00 a.m.) while the patient was fasting overnight.

Subjects receiving HPL decanoate were a group of clinically stabilized schizophrenic patients who were randomly assigned to receive a monthly dose of 200 ($n = 14$), 100 ($n = 17$), 50 ($n = 16$), and 25 ($n = 6$) mg of HPL decanoate intramuscularly for 12 months as maintenance therapy. Blood samples were drawn in the morn-

ing during weeks 4, 8, 12, 16, 20, 24, 28, 40, and 52 just before the administration of the next dose, with patients fasting overnight. All blood samples were centrifuged immediately at 4°C, plasma separated and stored at −60°C until analysis.

2.4. Extraction

To 1 ml of plasma taken in a 15-ml glass tube, 4 ng of ClHPL (100 μl of 40 ng/ml solution in acetonitrile) as an internal standard and 0.5 ml of saturated sodium carbonate were added and mixed well. Then the plasma was extracted with 7 ml of 10% methylene chloride in pentane by shaking in a Vibrax shaker for 10 min and subsequently the tubes were centrifuged at 18°C for 10 min. The supernatant liquid was transferred to a clean glass tube and evaporated to dryness in a heating block under a slow stream of nitrogen at 55°C. The residue was reconstituted in 150 μl of acetonitrile and an aliquot of this solution was injected onto the HPLC system.

2.5. Recovery

Absolute recoveries of HPL, RHPL and ClHPL were determined by extracting 1-ml aliquots of spiked plasma samples containing 15, 10, 5, 2.5, 1, 0.5, and 0.25 ng of HPL and RHPL per ml and 4 ng of internal standard and analysis by the method described here. The absolute recovery was calculated by comparing the peak heights obtained from extracts of spiked plasma samples and the peak heights obtained from direct injections of known amounts of standard solutions of HPL, RHPL and internal standard, ClHPL.

The concentrations of HPL and RHPL were calculated from the standard curve. The standard curve was constructed by plotting the peak-height ratio of HPL/ClHPL or RHPL/ClHPL on the y-axis and concentrations of HPL or RHPL, respectively on the x-axis. Spiked standard curve samples and quality control samples were subjected to the same experimental conditions and analyzed along with each batch of patients' plasma samples.

3. Results and discussion

The chromatograms of HPL, RHPL and ClHPL standards (1 ng) in acetonitrile are shown in Fig 1A. The chromatogram of the plasma from drug free volunteers (Fig. 1B) did not show any interfering compound extracted from the sample. A typical chromatogram of a drug free human plasma spiked with HPL, RHPL (3.5 ng/ml) and ClHPL (4 ng) is shown in Fig 1C. The chromatograms of the extract of plasma samples from patients receiving 40 mg/day of oral HPL and 200 mg/month of i.m. HPL decanoate are shown in Fig 2A and B, respec-

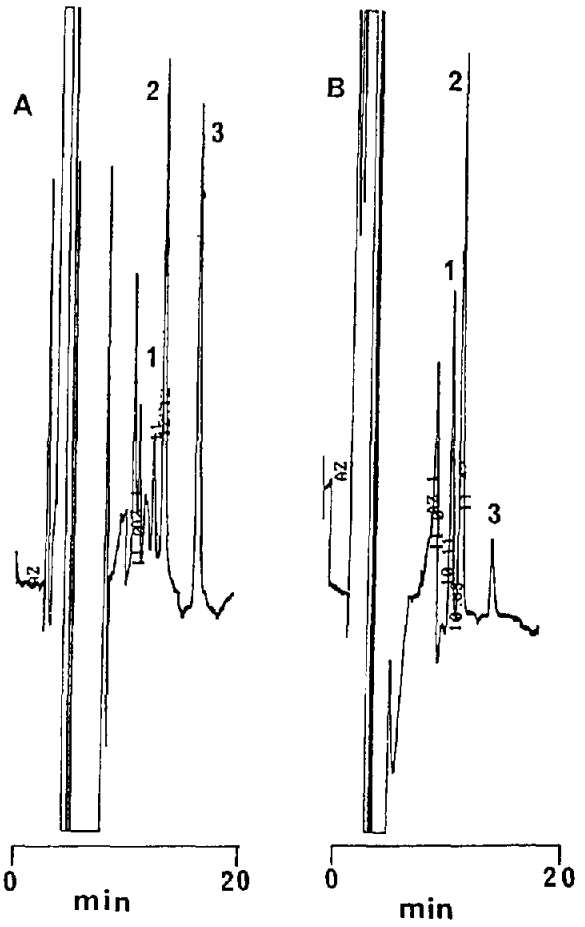

Fig. 2. Chromatograms of a plasma sample from patients receiving (A) 40 mg/day of oral HPL, and (B) 200 mg/month of i.m. HPL decanoate.

Table 1
Between-day and within-day assay variance of the HPLC method for HPL and RHPL

Concentrations added to blank plasma (ng/ml)	Experimentally determined concentration[a] (ng/ml)	
	HPL	RHPL
Between-day[b] *(n = 7)*		
7	7.09 ± 0.43 (5.7)	6.83 ± 0.28 (4.0)
3.5	3.5 ± 0.15 (4.1)	0.69 ± 0.24 (6.3)
0.7	0.71 ± 0.05 (5.9)	0.76 ± 0.05 (6.1)
0.35	0.36 ± 0.01 (3.9)	0.34 ± 0.03 (8.9)
Within-day[c] *(n = 6)*		
7	7.28 ± 0.15 (2.13)	6.99 ± 0.46 (6.5)
3.5	3.58 ± 0.12 (3.3)	3.84 ± 0.35 (9.2)
0.7	0.72 ± 0.04 (5.3)	0.66 ± 0.06 (8.5)
0.35	0.35 ± 0.01 (3.7)	0.28 ± 0.02 (7.5)
Patients' sample[d] *(n = 6)*		
I	15.12 ± 0.33 (2.3)	17.33 ± 0.64 (3.7)
II	3.6 ± 0.09 (2.5)	2.01 ± 0.09 (4.5)
III	5.27 ± 0.17 (3.3)	4.63 ± 0.42 (9.1)
IV	12.25 ± 0.2 (1.6)	21.3 ± 0.75 (3.5)

[a]Values in parenthesis are coefficients of variation (%).
[b]Between-day assay variance was calculated from the assay values obtained on seven different days of analysis of 1-ml aliquots of spiked plasma standards.
[c]Within-day assay variance was calculated from six different assay values obtained on a single day of analysis of 1-ml aliquots of spiked plasma standards.
[d]Four lots of pooled plasma samples from patients treated with oral HPL.

tively. The standard curves for HPL and RHPL were linear over the range 0.1–15 ng/ml and 0.25–15 ng/ml, respectively when 1 ml of plasma was used for analysis. The limit of quantitation for RHPL could be extended to 0.1 ng/ml when 2 ml of plasma was used for the analysis. Typical standard curves for HPL and RHPL could be defined by the equation $y = 0.3206x + 0.0007$ with a correlation coefficient of 0.9998 and $y = 0.1596x + 0.006$ with a correlation coefficient of 0.9987, respectively, where y is the peak-height ratio and x is the concentration (ng/ml) of HPL and RHPL.

The within-day (intra) and between-day (inter) assay variances are given in Table 1. The within-day assay variations were determined by analyzing six 1-ml aliquots of spiked plasma samples containing 7, 3.5, 0.7, and 0.35 ng of HPL and RHPL per ml and six aliquots of each of four lots of pooled plasma samples from patients treated with oral HPL. The between-day assay variations were determined by analyzing 1-ml aliquots of spiked plasma samples containing 7, 3.5, 0.7, and 0.35 ng of HPL and RHPL per ml on seven different days. In both cases the coefficient of variation was $< 10\%$ at all concentrations investigated. The absolute recovery of HPL, RHPL, and ClHPL by this extraction procedure is 68.6 ± 6.86 ($n = 53$), 86.1 ± 14.1 ($n = 64$), and 68.4 ± 8.26 ($n = 65$), respectively.

Table 2
Mean ± S.D. plasma concentration of HPL, RHPL and the ratios of RHPL/HPL in patients receiving oral doses of HPL

Oral dose (mg/day)	n[a]	Plasma concentration (ng/ml)		Ratio of RHPL/HPL
		HPL	RHPL	
80	5	52.34 ± 14.55	46.51 ± 21.26	0.91 ± 0.33
65	3	45.78 ± 16.13	16.95 ± 16.83	0.36 ± 0.30
60	6	29.46 ± 11.08	28.74 ± 22.72	0.74 ± 0.91
50	12	30.03 ± 9.92	25.61 ± 18.94	0.80 ± 0.53
35	10	18.41 ± 8.71	12.41 ± 12.95	0.60 ± 0.41
20	9	9.59 ± 2.95	3.7 ± 4.98	0.33 ± 0.29
15	7	8.38 ± 3.14	3.18 ± 3.34	0.36 ± 0.23
10	3	5.46 ± 2.54	1.99 ± 2.52	0.29 ± 0.22
5	4	2.26 ± 1.37	0.82 ± 0.38	0.40 ± 0.24

[a]n = number of patients.

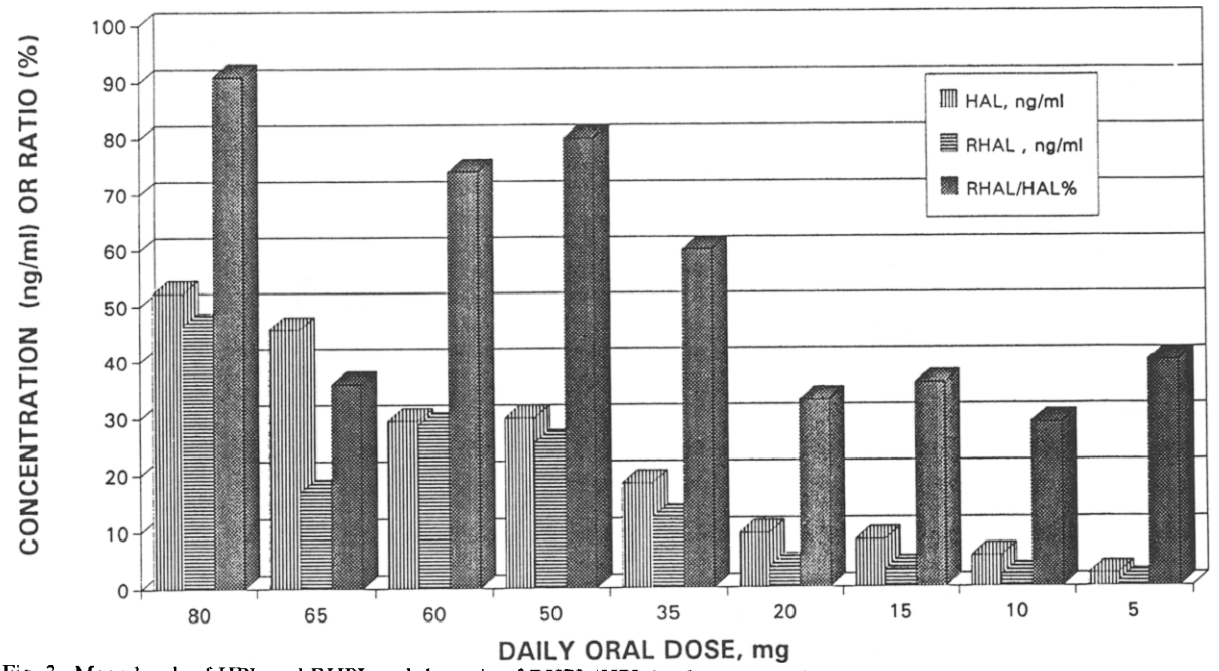

Fig. 3. Mean levels of HPL and RHPL and the ratio of RHPL/HPL in plasma of patients receiving daily oral doses ranging from 80 to 5 mg of HPL.

3.1. Application

The concentrations of HPL and RHPL in the plasma of patients were determined using the above described HPLC method. The overall mean plasma levels of HPL and RHPL and the ratios of RHPL/HPL in patients receiving 5 to 80 mg of oral HPL are given in the Table 2 and Fig. 3. The plasma levels of both HPL and RHPL were decreased with decreases in dose. However, the ratios of RHPL to HPL at doses of 35 to 80 mg/day were above 0.7 and they were similar except in the case of a 65 mg/day dose where the mean ratio was 0.36. This low ratio at the daily dose of 65 mg was observed because one of the patients receiving this dose had low levels of RHPL. Interestingly, the ratios of RHPL to HPL at doses lower than 35 mg/day were similar and consistently low.

The overall mean plasma levels of HPL and RHPL in patients receiving 200, 100, 50, and 25 mg of HPL decanoate per month as intramuscu-

Table 3
Mean ± S.D. plasma concentration of HPL, RHPL and the ratios of RHPL/HPL in patients receiving i.m. doses of HPL decanoate

Dose (i.m.) (mg/month)	n^a	Plasma concentration (ng/ml)		Ratio of RHPL/HPL
	HPL	RHPL		
200	107	2.67 ± 1.80	1.22 ± 1.89	0.36 ± 0.35
100	129	1.44 ± 0.95	0.54 ± 0.66	0.36 ± 0.46
50	127	1.11 ± 1.36	0.66 ± 0.95	0.37 ± 0.43
25	42	0.48 ± 0.29	0.27 ± 0.04	0.26 ± 0.06

[a] n = number of plasma samples.

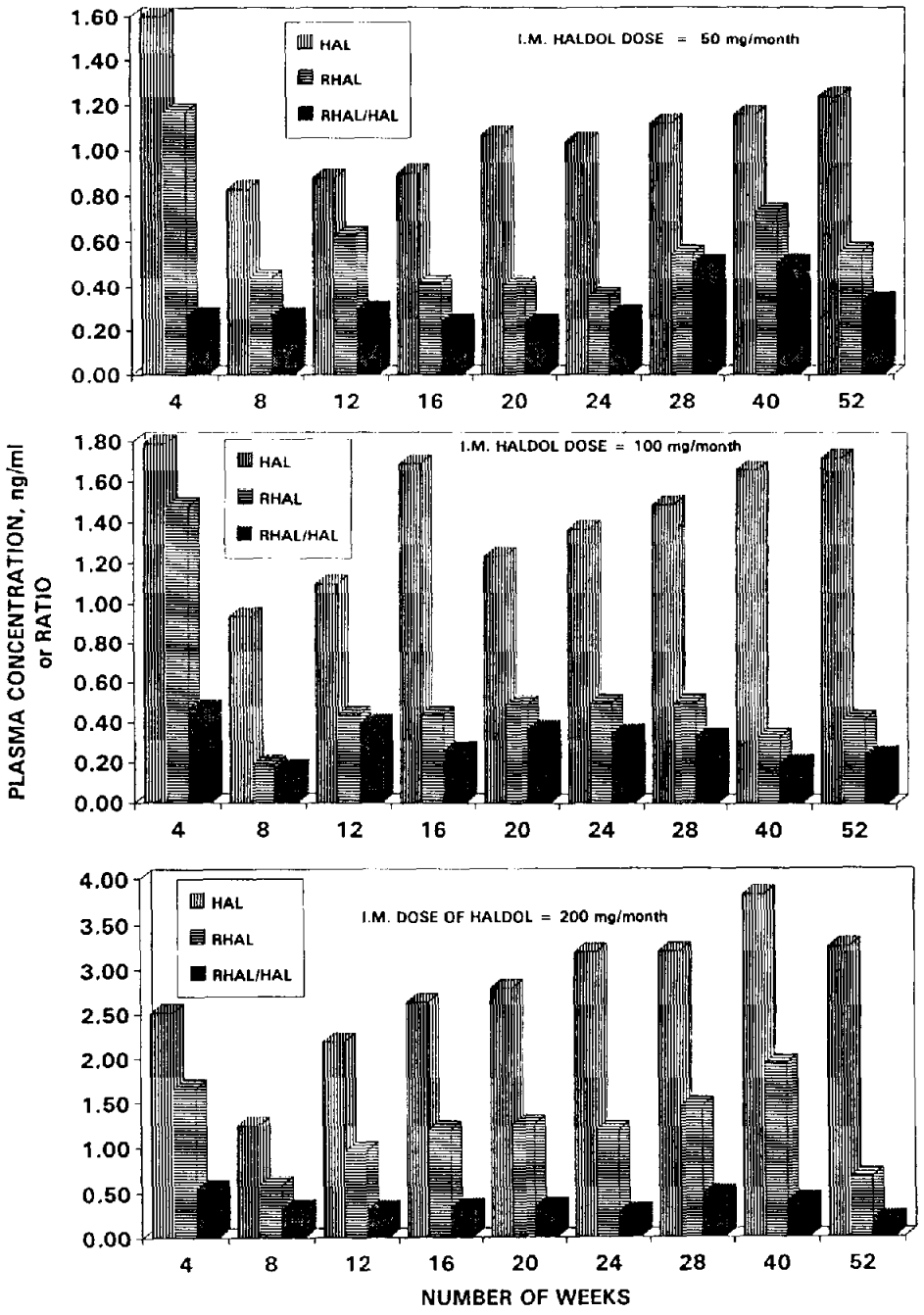

Fig. 4. Mean levels of HPL and RHPL and the ratio of RHPL/HPL in plasma of patients receiving monthly i.m. doses of HPL decanoate ranging from 50 to 200 mg over a period of 52 weeks.

lar injection are given in Table 3 and Fig. 4. HPL was present in measurable amounts in all but 5 of 42 samples from patients who received 25 mg, 1 of 127 samples from patients who received 50 mg and 3 of 129 samples from patients who received 100 mg/month dose of i.m. HPL. In the case of patients receiving a 200 mg/month dose, HPL was present in measurable levels in all plasma samples analyzed. However the metabolite RHPL was not present in all the plasma samples where HPL could be determined. In fact, RHPL was present in quantifiable levels in 38%, 63%, and 87% of the samples from patients receiving 50, 100, and 200 mg/month dose of i.m. HPL, respectively, in which HPL was determined. There was a proportional increase in the plasma levels of HPL and RHPL with an increase in i.m. dose. The ratio of RHPL/HPL is similar at all three doses studied ranging from 50 to 200 mg/month.

It is interesting to note that with a daily oral dose of 35 mg HPL and above, the inter-patient ratio of RHPL/HPL increased 2- to 3-fold as compared to that obtained after daily doses of 20 mg and below. In fact, a similar trend was noticed for the intra-patient ratio in patients who received a daily oral dose of HPL which was reduced from high to low in a step-wise manner. For these patients, the ratio of RHPL/HPL also decreased with the decrease in dose. However, when the dose was between 20 and 5 mg/day, the ratios became consistently low and similar even though the plasma concentrations continued to decrease with the decrease in daily HPL dose. This may be due to the fact that metabolic inter-conversion between HPL and RHPL is favored in the direction of RHPL as compared to RHPL to HPL [28] and hence RHPL tends to accumulate in the system when higher oral doses are administered. These ratios were similar to those obtained at all i.m. doses of HPL studied even though the determined concentrations of HPL and RHPL were much higher with low oral doses than at all i.m. doses. This may be due to the fact that in the case of i.m. doses, the administered HPL is not subjected to first pass metabolism by the liver and/or gut. Consequently, the metabolic conversion of HPL to RHPL is reduced, resulting in a similar RHPL/HPL ratio even when the i.m. dose was increased eight fold.

In summary, the described method for the simultaneous determination of HPL and RHPL in human plasma is simple and sensitive. This method is specific in that drugs generally co-administered did not interfere in the determination of HPL and RHPL. This method is being used in the routine determination of HPL and RHPL in the plasma of patients receiving oral or i.m. doses of HPL.

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