Pharmaceutical Research, Vol. 6, No. 12, 1989

Report

High Performance Liquid Chromatographic (HPLC) Determination of Centchroman in Human Serum and Application to Single-Dose Pharmacokinetics[1]

Jyoti Kumar Paliwal,[2] Ram Chandra Gupta,[2] Pyara Krishen Grover,[2,3] Omkar P. Asthana,[4] Jagdishwar Sahai Srivastava,[4] and Swaran NityaNand[4]

Received October 19, 1988; accepted June 7, 1989

A simple and sensitive (2 ng/ml) HPLC method with fluorescence detection has been developed to measure serum concentrations of centchroman, a new nonsteroidal antifertility agent. The method was sufficiently sensitive to follow the drug over 21 days in human volunteers. Pharmacokinetic parameters of centchroman were determined after a single oral dose of 60 mg (2 × 30-mg tablets) in two healthy female volunteers. Centchroman is slowly eliminated from serum, showing a biexponential disappearance curve from serum. The terminal half-life of centchroman in the two volunteers was 168 and 175 hr, respectively.

KEY WORDS: centchroman; high-performance liquid chromatography (HPLC); human pharmacokinetics.

INTRODUCTION

Centchroman (1), (1-2-[4-(3,4-dihydro-3,4-trans-7-methoxy-2,2-dimethyl-3-phenyl-2H-1-benzopyran-4-yl)-phenoxy] ethylpyrrolidine hydrochloride; Fig. 1) is a new nonsteroidal, once-a-week (2), orally effective, postcoital antifertility agent (3,4) in humans, which is also useful for the treatment of breast cancer (5). In the present study an HPLC method has been developed to determine concentrations of centchroman in human serum. The method has been successfully employed for the study of basic pharmacokinetic parameters of unchanged drug in normal healthy female volunteers after 60 mg (2 × 30-mg tablets) per oral dose. The blood samples were collected over 24 days after the dose. This study was limited to two volunteers because of the long sampling schedule.

MATERIALS AND METHODS

Reagents and Materials

Potassium dihydrogen orthophosphate, orthophosphoric acid, and acetonitrile were analytical-grade reagents. Acetonitrile was glass distilled before use. Diethyl ether (anesthetic grade ip) was used after purification. Centchroman free base (Pure reference standard) was supplied by the Pharmaceutics Division of this institute.

Extraction Procedure

Aliquots of serum (1–2 ml) were transferred into a 20-ml glass test tube 15 × 150 mm and extracted three times with 3-ml volumes of diethyl ether, after vortexing for 15 sec followed by centrifugation for 5 min at 1700 rpm. The ether layer was separated into a 15-ml conical glass centrifuge tube after snap-freezing the aqeous layer in liquid nitrogen. The combined ether extracts were evaporated to dryness under nitrogen at 35 ± 2°C, and the residue was reconstituted into 100 μl of methanol.

Apparatus and Chromatographic Parameters

A Kontron 600 liquid chromatograph equipped with a Shimadzu RF-530 variable-wavelength fluorescence detector was used. Peak area was integrated by a Shimadzu CR1B Chromatopac integrator-plotter. Separation was accomplished on a 4.6-mm-I.D. × 110-mm Spherisorb cyano 5-μm cartridge analytical column with a 4.6-mm-I.D. × 30-mm cyano 10-μm guard column (Kontron AG, Switzerland). The mobile-phase flow rate was 1 ml/min and the fluorescence detector was set at 279-nm excitation and 305 nm-emission wavelengths. Quantitation was done by the external standard method.

The mobile phase was prepared by mixing acetonitrile, 10 m*M* potassium dihydrogen orthophosphate, and 0.3 m*M* orthophosphoric acid at a ratio of 60:32:8, respectively. It was filtered and degassed before use.

[1] CDRI Communication No. 4377.

[2] Pharmacokinetics and Metabolism Division, Central Drug Research Institute, Lucknow-226001, India.

[3] To whom correspondence should be addressed.

[4] Clinical and Experimental Medicine Division, Central Drug Research Institute, Lucknow-226001, India.

0724-8741/89/1200-1048$06.00/0

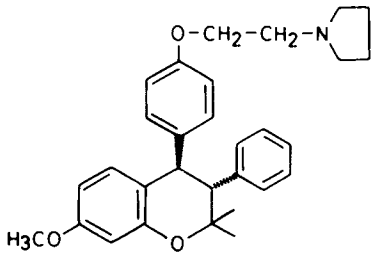

Fig. 1. Centchroman.

Calibration Graph

Standard solutions containing 30, 40, 60, 80, 100, 200, 400, 600, and 800 ng/ml of centchroman in mobile phase were prepared by dilution of a 15 μg/ml methanolic stock solution. Each standard solution was analyzed on HPLC five times and a calibration curve was obtained using the mean area of the five values at each point.

Extraction Recovery and Assay Reproducibility

The recovery of centchroman from serum was determined at three different concentrations. Known amounts of centchroman were added to drug-free serum and the area responses of centchroman in extracted spiked samples were compared with those obtained by direct injection of standard solutions containing equivalent amounts of centchroman.

Assay reproducibility was assessed at low, medium, and high concentrations of centchroman. Within-day reproducibility was calculated from five replicate analysis. Day-to-day reproducibility was determined from duplicate analysis on 3 different days.

Subjects

Approval for the study was obtained from the local ethical committee and was in accordance with the regulations of the drug controller of India. Two healthy females aged 39 and 41 years and with body weights of 62 and 52 kg, respectively, participated in this study after providing written informed consent. These subjects were selected for study after passing a physical examination demonstrating no evidence of disease and after passing a laboratory survey consisting of hematocrit, red and white blood cell counts, differential platelet estimate, SGOT, SGPT, serum bilirubin, serum urea, fasting serum glucose, serum creatinine, serum cholesterol, serum albumin, and serum globulin. All the tests were repeated after 21 days. Pulse rate and BP were also monitored prior and up to 8 hr post centchroman administration.

Drug Administration

Each subject was given a single 60-mg (2 × 30-mg tablets) dose orally in the morning with 250 ml of water. Food and fluids were allowed 2 hr after the oral dose.

Blood Samples

Blood samples (10 ml) were drawn at 0, 0.5, 1, 2, 4, 6, 8, 12, 24, 36, 48, 72, 96, 120, 168, 240, 336, and 504 hr after drug administration. Samples up to 12 hr were drawn from the anterior cubital vein by a long indwelling Becton–Dickinson intravenous catheter which was implanted into the forearm vein prior to the start of the study. Later samples were collected by venipuncture in glass tubes. Serum was separated and stored at −20°C in glass tubes until analyzed.

Pharmacokinetic Data Analysis

Pharmacokinetic analysis of serum concentration–time data was done on an IBM PC/XT microcomputer using the statistical package STATIS2 (6). Initial parameter estimates for nonlinear regression analysis were obtained by a curve stripping program, ESTRIP (7). The criteria (F test) discussed by Boxenbaum and co-workers (8), Akaike's information criteria (9), and Schwarz criteria (10) were used in deciding on the most appropriate model describing the concentration–time data. In addition, the residuals (observed concentration − calculated concentration) were examined using the run test to check for bias in curve fitting (11). A two-compartment open model with first-order absorption and elimination rate constants gave the best description of data. The following equation has been employed to describe the time course of such a drug in the body (see Ref. 12).

$$C = A_1 e^{-\alpha(t-t_0)} + A_2 e^{-\beta(t-t_0)} - (A_1 + A_2) e^{-K_a(t-t_0)}$$

where

K_a = first-order absorption rate constant,
α and β = apparent first-order fast and slow disposition rate constants,
A_1 and A_2 = corresponding zero-time intercepts,
C = concentration of drug in serum at any time t,
t_0 = time lag.

Maximum plasma concentration (C_{max}) and time to reach the maximum (T_{max}) were determined from the experimental points. Total apparent oral clearance of the drug (Cl_{tot}/F) was calculated as D/AUC (0–∞), where D is the oral dose and AUC (0–∞) is the area under the serum concentration–time curve extrapolated from 0 to infinity. Apparent volume of distribution (V_d/F) was calculated as $V_d/F = (Cl_{tot}/F)$. The half-life ($t_{1/2}$) was calculated from the quotient as $t_{1/2}$ = 0.693/corresponding slope.

RESULTS AND DISCUSSION

Chromatography

Figure 2 illustrates a typical chromatogram of serum from a normal and a centchroman-receiving volunteer. Under the chromatographic conditions the retention time of centchroman was 6.2 min. Extracted serum constituents did not interfere since they eluted either at the solvent front or after the peak of interest.

Absolute recoveries (mean ± SD) of centchroman from human serum using the described extraction procedure were 95 ± 4.8, 96 ± 3.2, and 96 ± 2.7 at 2, 50, and 150 ng/ml levels, respectively.

Reproducibility was excellent for both within-day and day-to-day analysis. Coefficients of variation for within-day analysis (N = 5) were 1.5% at 150 ng/ml, 3.7% at 50 ng/ml,

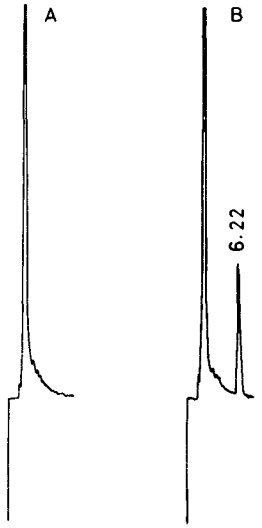

Fig. 2. High-performance liquid chromatograms of (A) blank human serum and (B) centchroman in serum.

and 7.9% at 2 ng/ml. The corresponding values for day-to-day analysis (N = 6) were 2.3, 5.7, and 9%.

A calibration curve of centchroman in standard solutions was plotted each day before sample analysis. Linear least-squares regression analysis of the calibration graph demonstrated linearity between the peak area response and the corresponding centchroman concentration in the examined concentration range of 30 to 800 ng/ml of drug. A typical standard curve could be described by $y = 6.988\,x + 7.413$ (r = 0.9999).

The limit of the assay sensitivity was 30 ng/ml in standard solution and 2 ng/ml in serum samples (when 2 ml of serum was extracted and reconstituted in 100 μl), with an acceptable precision as indicated by within-day and day-to-day analysis variations.

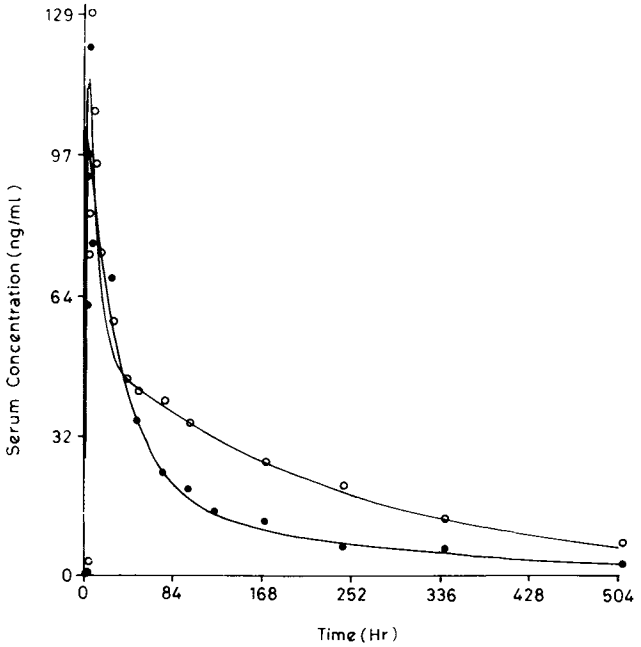

Fig. 3. Serum levels of centchroman in two volunteers after a single 60-mg oral dose.

Table I. Pharmacokinetic Parameters of Centchroman in Two Volunteers After a Single Oral Dose of 60 mg (2× 30-mg Tablets)

Parameter	Volunteer SM	Volunteer SS
A_1 (ng/ml)	124	91.7
A_2 (ng/ml)	52.9	19.7
K_a (L/hr)	0.777	1.78
α (L/hr)	0.133	0.0305
β (L/hr)	0.00412	0.00397
t_0 (hr)	0.411	0.0237
$t_{1/2}$ absorption (hr)	0.892	0.389
$t_{1/2}$ α (hr)	5.21	22.72
$t_{1/2}$ β (hr)	168.2	174.56
AUC (0–∞) (ng/ml • hr)	13500	7910
Cl/F (L/hr)	4.44	7.58
V_d/F (L)	1077	1909
T_{max} (hr)	4	4
C_{max} (ng)	129	121

Pharmacokinetics

Pharmacokinetics of centchroman was studied in two normal healthy female subjects. Normal status prior to and at the end of study was ensured by physical and laboratory investigations. Figure 3 depicts the serum time course of centchroman in two subjects after a single dose of 60 mg (2 × 30-mg tablets) orally. Table I contains the estimated pharmacokinetic parameters. Serum concentration–time data for both the volunteers were best described by a two-compartment open model with first-order absorption and elimination rate constants. There is a good agreement between the calculated and the actual serum concentrations of the drug. A time lag of 0.41 and 0.02 hr was needed to fit the data, indicating a delayed absorption of drug from GI tract. The half-life of absorption was 0.77 and 1.78 hr, respectively. Goodness of fit of the data to the selected equation is indicated by R values of 0.981 and 0.980, respectively.

The terminal disposition half-life of drug was calculated to be 168 and 174 hr (approximately 7 days). Maximum serum concentrations of centchroman were 121 and 129 ng, respectively, at 4 hr. Absolute bioavailability of centchroman in serum could not be determined due to nonavailability of its parenteral formulation. The apparent oral clearance (Cl/F) and F volume of distribution (V_d/F) in Table I are the measured values divided by the bioavailability (F). Centchroman appears to be extensively distributed in the tissues as evidenced by the high values of the apparent volume of distribution.

The terminal centchroman half-life of 7 days suggests that it can be used as a once-a-week oral contraceptive. It may thus be more readily accepted than steroid contraceptives which have to be taken daily.

ACKNOWLEDGMENTS

The authors thank Dr. M. M. Dhar Ex. Director for his

constant encouragement, Mr. T. B. Singh for technical assistance, and Mr. Tej Singh for typing the manuscript.

REFERENCES

1. S. Ray, P. K. Grover, V. P. Kamboj, B. S. Setty, A. B. Kar, and N. Anand. *J. Med. Chem.* 19:276–279 (1976).
2. N. Anand. *Proc. Ind. Natl. Sci. Acad.* 49A:233 (1983).
3. N. Anand and S. Ray. *Ind. J. Exp. Biol.* 15:1142–1143 (1977).
4. V. P. Kamboj. Proceedings of the joint symposium organised by ICMR and WHO, Banglore (1985).
5. N. C. Misra, D. Kumar, A. K. Agarwal, B. S. Setty, S. S. Agarwal, S. Ray, and V. P. Kamboj. 13th International Congress on Cancer, Seattle, 1982, Abstract 3033.
6. A. W. Kelman. Statis2 (Version 2.1) Statistics package, Stobhill General Hospital, Glasgow G21 3UW.
7. R. D. Brown and J. E. Manno. *J. Pharm. Sci.* 67:1687–1691 (1978).
8. H. G. Boxenbaum, S. Riegelman, and R. M. Elasho. *J. Pharmacokin. Biopharm.* 2:123–148 (1974).
9. K. Yamaoka, T. Nakagawa, and T. Uno. *J. Pharmacokin. Biopharm.* 6:165–175 (1978).
10. G. Schwarz. *Ann. Stat.* 6:461–464 (1978).
11. N. R. Darper and H. Smith. *Applied Regression Analysis,* John Wiley and Sons, New York, 1975, pp. 95–99.
12. P. G. Welling. *Pharmacokinetics Process and Mathematics,* ACS Monograph 185, American Chemical Society, Washington, D.C., 1986, pp. 229–237.