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Note

Sensitive high-performance liquid chromatographic assay of pelanserin, a novel antihypertensive agent, in plasma samples

F.J. FLORES-MURRIETA, E. HONG and G. CASTAÑEDA-HERNÁNDEZ*

Sección de Terapéutica Experimental, Departamento de Farmacología y Toxicología, Centro de Investigación y de Estudios Avanzados del I.P.N., Apartado Postal 22026, 14000 Mexico, D.F. (Mexico)

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Pelanserin, 2,4-(1*H*,3*H*)-quinazolinedione-3-[3-(4-phenyl-1-piperazinyl)-propyl] hydrochloride (I, Fig. 1), is an effective agent for the treatment of arterial hypertension [1]. Pelanserin is a potent antagonist of S_2 receptors [2–4], and possesses α_1 blocking properties [2]. Additionally, it has been reported that pelanserin has a vasodilator effect not related to the above-mentioned properties, probably mediated by calcium entry blockade [5].

To date, only limited information is available concerning the pharmacokinetics of this drug [6]. Evaluation of pharmacokinetic data and plasma level monitoring will certainly contribute to a better understanding of the therapeutic efficacy of pelanserin and will provide a rational basis for the establishment of proper dosage regimens of this new drug. This paper describes a sensitive assay for pelanserin in plasma and the verification of its use in measuring plasma levels in humans after therapeutic doses.

EXPERIMENTAL

Chemicals and solutions

Pelanserin and the internal standard, 2,4-(1*H*,3*H*)-quinazolinedione-1-methyl-3-[2-(4-phenyl-1-piperazinyl)ethyl] maleate (II, Fig. 1), were obtained from the Sección de Terapéutica Experimental, CINVESTAV-IPN (Mexico City, Mexico). Acetonitrile was chromatographic grade (E. Merck, Darmstadt, F.R.G.). Deionized water was prepared using a Milli-Q reagent water system (Continental Water Systems, El Paso, TX, U.S.A.). All other reagents were of analytical grade.

Stock solutions of pelanserin and of the internal standard corresponding to

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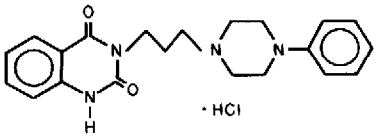
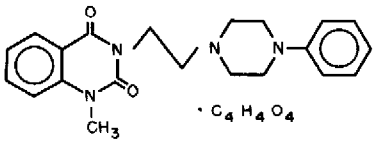


I



II

Fig. 1. Structures of pelanserin (I) and of the internal standard (II).

free base concentrations of 0.912 and 0.760 mg/ml were prepared in mobile phase (see below). These solutions were stable at least for six months when stored at −4 °C. Standard solutions of pelanserin were obtained by diluting the stock solution to free base concentrations ranging from 91.2 to 4560 ng/ml in mobile phase. The standard solution of the internal standard was prepared at a free base concentration of 760 ng/ml.

The following aqueous solutions were prepared: 0.01 *M* sodium dihydrogenphosphate, pH adjusted to 6.0 with 0.01 *M* dipotassium hydrogenphosphate; 2 *M* sodium carbonate, pH adjdusted to 12.0 with 1 *M* sodium hydroxide.

Sample preparation

Plasma samples of 2 ml (unknown samples, drug-free plasma or plasma standards containing known amounts of pelanserin) were pipetted into conical glass tubes and spiked with 200 μl of a 760 ng/ml solution of II (152 ng of free base). After the addition of 0.25 ml of a 2 *M* sodium carbonate buffer (pH 12.0), the alkalinized plasma was extracted with 4 ml of pentane–ethyl acetate (3:1) using a Yankee variable-speed rotator (Clay Adams, Parsippany, NJ, U.S.A.) set at 60 rpm. The two phases were separated by centrifugation at 3000 *g* for 5 min. The upper organic layer was transferred to another conical glass tube, and the plasma was extracted again with 4 ml of pentane–ethyl acetate. The combined organic layers were evaporated to dryness at 45 °C under a gentle stream of nitrogen. The dry residue was reconstituted with 200 μl of mobile phase (see below) by vigorous vortexing, and 80-μl aliquots were injected into the chromatographic system.

Chromatographic conditions

The high-performance liquid chromatographic (HPLC) system was manufactured by Waters Assoc. (Milford, MA, U.S.A.). It consisted of two solvent-delivery systems (Models 510 and M-45), an automatic gradient controller (Model 680), an universal injector (Model U6K), a programmable multiwavelength detector (Model 490) and a data module (Model 730). Analyses were performed on a Novapak C_{18} (150 mm × 3.9 mm I.D., particle size 5 μm) column using aceto-

nitrile–0.01 *M* phosphate buffer (pH 6.0) (1:2, v/v) as the mobile phase. The column was kept at room temperature (20–24°C). The flow-rate was kept constant at 1.5 ml/min. The absorbance of the column effluent at 244 nm was recorded at a sensitivity of 0.005 a.u.f.s. To prolongue the life of the analytical column, a precolumn (25 mm×3.9 mm I.D.) containing 37–50 μm Corasil C_{18} (Waters) was incorporated into the system.

After a 17-min run with the mobile phase, the column was washed with 100% acetonitrile for 1 min. Then, a concave gradient was performed in order to return to the mobile phase composition in 2.2 min. With this procedure the elution of any endogenous compounds remaining in the column was achieved, allowing the injection of samples every 25 min.

Assay calibration

The assay was calibrated by addition of known amounts of pelanserin and internal standard to blank control plasma samples (2 ml). Calibration curves were established for pelanserin free base concentrations ranging from 9.12 to 456.0 ng/ml. The internal standard was used at a fixed free base concentration of 76.0 ng/ml.

The final sample concentrations were calculated by determination of the peak-height ratios of pelanserin related to the internal standard.

RESULTS

Typical chromatograms of extracted plasma samples are shown in Fig. 2. Retention times for pelanserin and for the internal standard were 7.5 and 14.0 min, respectively. No interfering peaks occurred at these times. However, when plasma samples containing pelanserin concentrations higher than 91.2 ng/ml were assayed, it was observed that the pelanserin peak had a shoulder caused by fusion with a small peak occurring at 8.7 min and corresponding to an unidentified endogenous compound (Fig. 2C). This shoulder did not significantly affect the determination of the pelanserin peak height. A linear relationship ($r=0.9998$) was found when the ratio of the peak height of pelanserin to the peak height of the internal standard was plotted on the y-axis against various concentrations of pelanserin (free base) ranging from 9.12 to 456.0 ng/ml on the x-axis. The equation by the least-sqaures method was $y=0.0113x+0.0615$.

The recovery of pelanserin from plasma samples following extraction, assessed by comparison of peak heights from plasma extracts with those from standard solutions, ranged from 85 to 95%. The accuracy of the assay was ascertained by adding known pelanserin amounts to drug-free plasma and analysing the samples with the method described. The results are summarized in Table I.

The reproducibility of the assay was determined by replicate analyses of spiked plasma samples. The intra-assay coefficient of variation was 4.7% at a concentration of 18.24 ng/ml ($n=10$) and 2.4% at a concentration of 91.20 ng/ml ($n=6$). Day-to-day precision was determined over a period of three weeks. Coefficients of variation were 7.8% at 18.24 ng/ml ($n=7$) and 4.7% at 91.20 ng/ml ($n=7$). The detection limit (signal-to-noise ratio = 2) was 3 ng/ml.

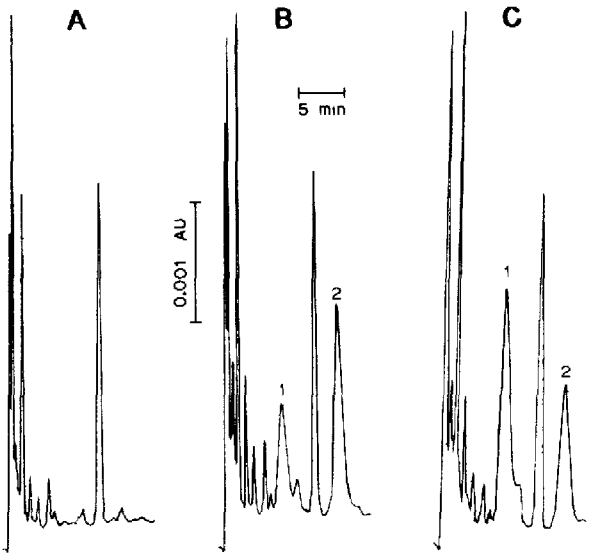


Fig. 2. Chromatograms of human plasma extracts. (A) Blank plasma. (B) Spiked plasma containing 45.6 ng/ml pelanserin (1) and 76.0 ng/ml internal standard (2). (C) Plasma from a healthy volunteer 120 min after a 20-mg oral dose spiked with 76.0 ng/ml internal standard.

TABLE I

ACCURACY OF THE HPLC METHOD FOR THE DETERMINATION OF PELANSERIN IN PLASMA SAMPLES

Theoretical concentration (ng/ml)	Observed concentration (mean ± S.E.M., $n=5$) (ng/ml)	Accuracy (%)
18.24	16.14 ± 0.38	88.5
45.6	43.25 ± 1.32	94.8
68.4	71.97 ± 1.43	105.2
91.2	92.52 ± 1.89	101.4
228	232.96 ± 5.76	102.2
456	452.98 ± 17.77	99.3

To demonstrate the effectiveness of the method for pharmacokinetic studies, blood samples from healthy volunteers were drawn following the administration of a 20-mg pelanserin capsule. Fig. 3 depicts a representative plasma concentration–time profile, indicating that the assay is suitable for pharmacokinetic and clinical studies. The assay has also proved to be valuable for the determination of pelanserin plasma levels in dogs after either intravenous or oral administration. Other widely used antihypertensive drugs, such as propranolol, hydrochlorotiazide, chlorotiazide, hydralazine, nifedipine and prazosin, did not interfere with the chromatographic determination of pelanserin.

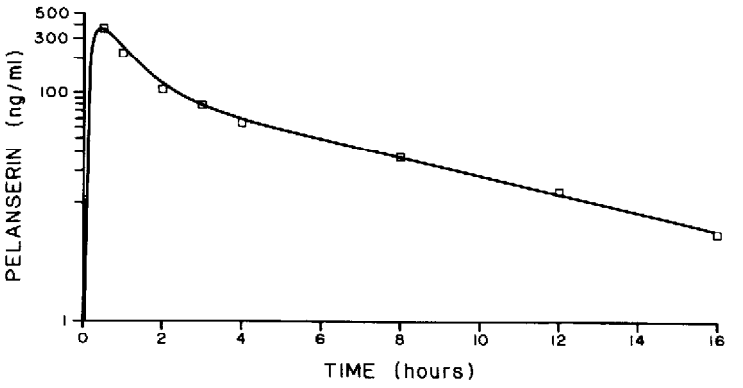


Fig. 3. Plasma concentration–time profile of pelanserin in a healthy volunteer following a single oral dose of 20 mg. The calculated pharmacokinetic parameters in this particular subject were: elimination half-life ($t_{1/2\beta}$), 3.76 h; distribution half-life ($t_{1/2\alpha}$), 0.53 h; absorption rate constant (k_a), 2.9 h^{-1}; intercept of monoexponential α (A), 451.18 ng/ml; intercept of monoexponential β (B), 119.65 ng/ml. Calculations were made on the basis of a two-compartment model by the exponential residual method.

DISCUSSION

Several solvent systems were assayed for the extraction of pelanserin from plasma samples. It has been reported that heptane–*tert.*-amyl alcohol (92:8) is a suitable extractant for ketanserin [7], a molecule structurally related to pelanserin. This solvent system was also efficient for the extraction of pelanserin; however, a time-consuming back-extraction step was required in order to eliminate interfering endogenous compounds. Therefore, a mixture of pentane–ethyl acetate (3:1) was selected since it proved to be efficient (recovery above 85%) and, although several endogenous compounds were co-extracted, there was minimal interference with the chromatographic determination of pelanserin. Co-extractants remaining in the column after the elution of the internal standard were washed out with 100% acetonitrile. It was found that this procedure was less tedious than a back-extraction step.

Maximum absorbance of pelanserin in mobile phase occurred at 220 nm. However, detection was carried out at 244 nm, the secondary absorbance peak, since the signal-to-noise ratio was significantly lower.

The HPLC method described has been demonstrated to be sufficiently sensitive and precise for the determination of pelanserin plasma levels in the clinically relevant concentration range. The entire procedure can be easily carried out by one person, and over 25 plasma samples can be assayed daily. This method is therefore adequate for the performance of pharmacokinetic studies of pelanserin in humans.

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