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Short communication

Determination of tezosentan, a parenteral endothelin receptor antagonist, in human plasma by liquid chromatography–tandem mass spectrometry

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Abstract

An analytical method was developed for the quantification of tezosentan in human plasma obtained in clinical studies. The method was linear in the range 1 to 512 ng/ml. After liquid–liquid extraction, the samples were analyzed by reversed-phase HPLC with tandem mass spectrometry. The limit of quantification was 1 ng/ml and the extraction recovery was at least 88.2%. Intra- and inter-assay coefficients of variation were below 10%. Stability tests revealed that tezosentan is stable under the different conditions tested.

Keywords: Tezosentan

1. Introduction

Endothelin-1 (ET-1) is a potent and long-lasting vasoconstrictor [1]. Furthermore, ET-1 is involved in bronchoconstriction and also plays a role in neurotransmitter release, inflammation, cell proliferation and fibrosis. It exerts its actions via two characterized ET receptors, ET_A and ET_B. These receptors are very similar in structure and belong to the superfamily of G protein-coupled receptors [2].

Tezosentan is a new, non-peptidic, ET receptor antagonist with high affinity for both ET_A and ET_B receptors [3]. It is specifically designed for parenteral use. Tezosentan is in clinical development for the treatment of acute heart failure [4,5]. The pharmacokinetics of tezosentan in healthy subjects have recently been described [6,7].

No methods have been described to quantify tezosentan in biological matrices. The purpose of the present study was to develop a rapid, reproducible, reliable and selective chromatographic method for the determination of tezosentan in human plasma. The developed method is based on a previously reported method [8] for the determination of bosentan, a compound related in structure to tezosentan.

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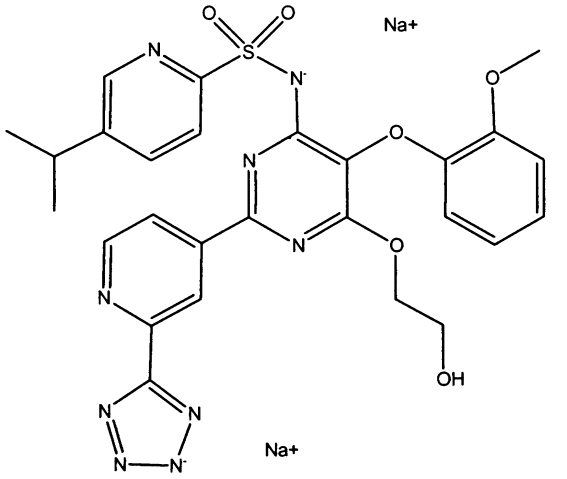
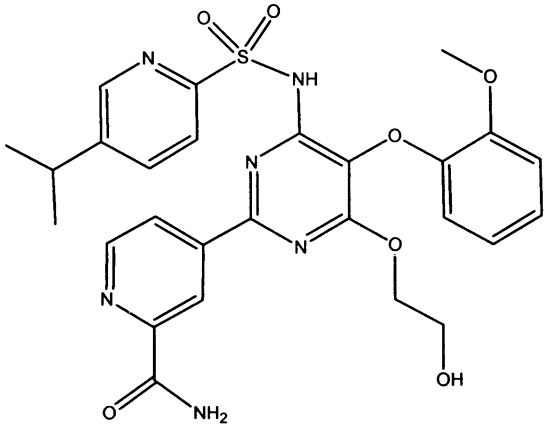
2. Experimental

2.1. Materials

Tezosentan and the internal standard (I.S.), Ro 64-0315, (for structures, see Fig. 1) were obtained from F. Hoffmann-La Roche (Basel, Switzerland). Purified HPLC water was obtained from Biosolve (Valkenswaard, Netherlands) and all other chemicals were of analytical grade obtained from commercial sources.



Tezosentan



Internal standard, Ro 64-0315

Fig. 1. Chemical structures of tezosentan and the I.S.

2.2. Instrumentation

The chromatographic system consisted of three pumps. Two LC-10ADvp pumps (Shimadzu, Kyoto, Japan) were used to elute the guard and analytical column with solvents A and B. The third pump, TSP P2000 (TSP, Allschwil, Switzerland), was used to wash the guard column with solvent C. Solvent A consisted of water–formic acid (99:1, v/v), solvent B of acetonitrile–formic acid (99:1, v/v) and solvent C of acetonitrile–water–formic acid (90:9:1, v/v). All three solvents were degassed with an SDU 2004 degasser (LabSource, Reinach, Switzerland). The column-switching system consisted of a 2.1×20 mm Haipeek Clipeus Phenyl (5.0 μm particle size, 120 Å pore size) guard column (Higgins Analytical, Mountain View, CA, USA) and a 2.1×50 mm Symmetry C_{18} (3.5 μm particle size, 100 Å pore size) analytical column (Waters, Rupperswil, Switzerland), which were separated by a six-port switching valve (LabSource). Both columns were placed in an LC-Pelcooler oven (LabSource) at 50 °C. Samples were placed on a HTS PAL autosampler (CTC Analytics, Zwingen, Switzerland) maintained at 4 °C by means of a Peltier cooler tray (LabSource) and 40 μl were injected into a 100-μl injection loop which was connected to the guard column. After injection, the guard and analytical columns were eluted for 30 s with a mixture of solvent A–solvent B (9:1, v/v), for 90 s with solvent A–solvent B (6:4, v/v) and for 90 s with solvent A–solvent B (4:6, v/v) at a flow-rate of 200 μl/min. The valve was then switched to allow back-flush of the guard column with solvent C for 90 s at a flow-rate of 200 μl/min in order to preserve the analytical column. Subsequently, guard and analytical columns were washed for 90 s with solvent A–solvent B (9:1, v/v, 200 μl/min).

Mass spectrometric detection was performed with an API 365 triple-quadrupole mass spectrometer from Perkin-Elmer Sciex (Concord, Ontario, Canada) operating in electrospray ionization positive ion mode, and equipped with a Turbolon spray ion source (350 °C). The collision energy and pressure were 25.8 eV and $2.1 \cdot 10^{-5}$ Torr, respectively, the dwell times for tezosentan and the I.S. were 300 and 50 ms, respectively, and the quadrupole resolution was 0.70±0.15 amu of the half-peak intensity.

Detection and quantification were performed using the standard software supplied in selected reaction monitoring mode. Precursor and product ion fragments used for quantification of tezosentan were at *m/z* 606→578 and for Ro 64-0315 at *m/z* 581→537. Samples were quantified using peak area ratios. Sample list, the acquisition method and data collection were performed on a Macintosh Power PC 7500 computer using Sample Control software (version 1.3) from Perkin-Elmer Sciex. The acquired data were processed with MacQuan software (version 1.5, Perkin-Elmer Sciex).

2.3. Sample preparation

Frozen samples were thawed in a cold-water bath and subsequently centrifuged (2790 *g* for 20 min at 4 °C). An aliquot of 0.5 ml plasma was transferred into another tube and 50 μl of 1 *M* sodium hydroxide were added in order to denaturate plasma proteins to which tezosentan is extensively bound [9]. This step greatly improved the recovery. After mixing for 15 s and centrifugation at 20 160 *g*, 1 ml of I.S. solution (500 ng/ml in methyl *tert.*-butyl ether) and 125 μl of 1 *M* orthophosphoric acid were added. Acidification was necessary to allow extraction of the weak acid tezosentan into the organic phase. The samples were extensively mixed, centrifuged at 20 160 *g* for 3 min at room temperature and subsequently kept at −80 °C for at least 20 min, which allowed for a better separation of the two phases. The organic phase (0.7 ml) was transferred into another tube and evaporated to dryness by means of an IR-Dancer device (Prolab, Reinach, Switzerland). After reconstitution of the sample in 150 μl methanol–water (1:9, v/v) containing 0.25% (v/v) ammonia (25%), 40 μl were injected onto the column-switching HPLC system.

2.4. Assay qualification

Several plasma samples obtained by pooling of pre-dose plasma samples from different healthy subjects were tested for the absence of interfering peaks. Stock solutions (0.512 mg/ml) of tezosentan were prepared in methanol–water (1:1, v/v) with 0.2% (v/v) of 25% ammonia solution. Working solutions for the preparation of calibration and quality control samples were prepared by dilution of the stock solutions with methanol–water (1:1, v/v). Human blank plasma was spiked with 1% (v/v) of these working solutions in order to obtain calibration samples of 1–512 ng/ml and quality control samples of 1, 3, 60, and 420 ng/ml. These plasma standards were used to determine the intra-day and inter-day variability. For this, six replicates of each quality control concentration were determined on a single day. Inter-day variability was determined by measuring the concentration of a set of quality control samples spiked with 1, 3, 60, and 420 ng/ml of drug on 4 different days. Precision and accuracy were calculated using standard methods. The LOQ was determined using quality control samples and defined as the lowest concentration at which the signal-to-noise ratio was greater than 10 and values for imprecision and inaccuracy were less than or equal to 20%. The absolute recovery was determined by comparing extracted spiked plasma samples with samples with the same concentration prepared in methanol–water (1:1, v/v). Furthermore, a number of tests were performed to check the stability of tezosentan in the stock solution assessed over 3 months at 6 °C, after short-term storage (6 h) at room temperature, frozen in plasma for 21 months at −20 °C, after three cycles of repeated thawing–freezing (at −20 °C), and after storage of extracted samples in the autosampler at 4 °C for 3 days. For these stability tests, quality control samples at 3 and 420 ng/ml were used. Stored samples were compared with freshly spiked samples. When stored samples deviated by less than 15% from freshly spiked samples, tezosentan was considered stable.

3. Results

Under the chromatographic conditions used, tezosentan (Fig. 2) and Ro 64-0315 (retention time 4 min for both) gave fully resolved and essentially symmetrical peaks. Total run time per sample was 6.5 min.

The peak area of tezosentan varied linearly with concentration over the range tested. The correlation coefficients (*r*) of the calibration curves were equal to or greater than 0.997. The parameters of the calibration curves obtained in this study are as

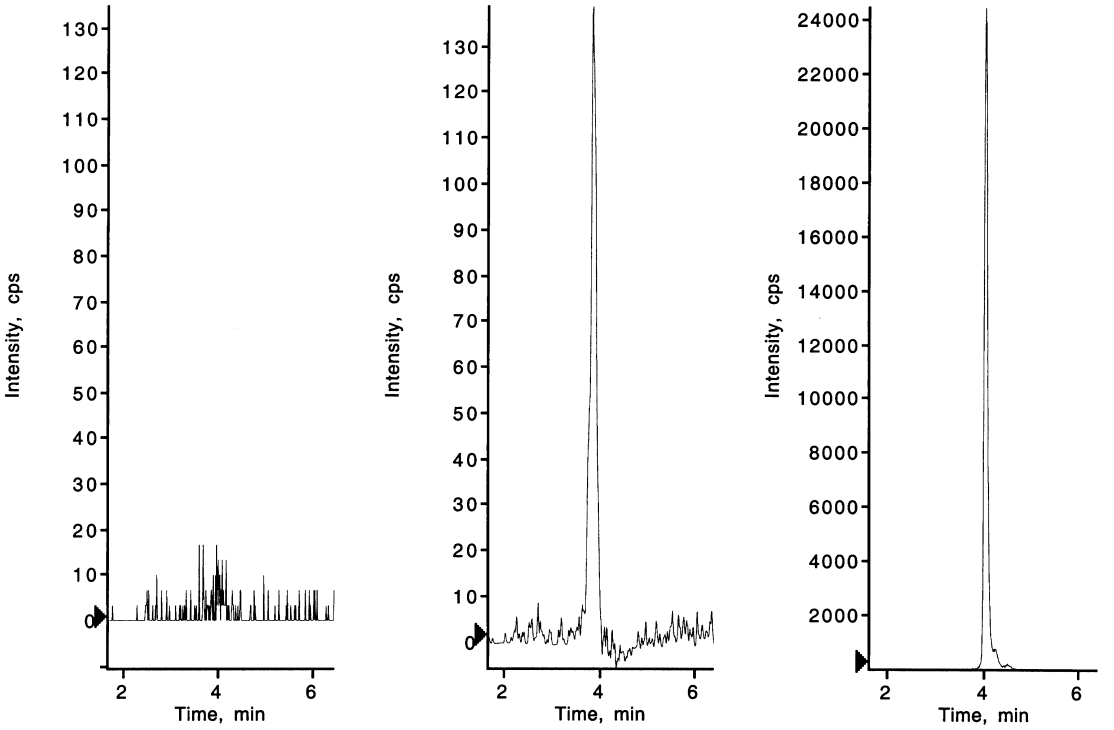


Fig. 2. Chromatograms of a blank human plasma sample (left), a human plasma sample spiked with 1 ng/ml tezosentan (center), and a human plasma sample with a measured concentration of 148 ng/ml from a healthy subject obtained 30 min after termination of an intravenous infusion of 50 mg/h of tezosentan for 1 h (right).

follows: correlation coefficient, 0.9975±0.00058; slope, 3.13±0.34; intercept, 0.0005±0.00058 (n=4). The limit of quantification was set at 1 ng/ml. The intra- and inter-day inaccuracy and imprecision data calculated from independently measured QC samples are shown in Table 1. All values for imprecision and inaccuracy were below 10 and 15%, respectively.

The extraction recovery was 88.2, 90.3 and 96.7% at levels of 3, 60 and 420 ng/ml, respectively. These results suggest that the extraction recovery is independent of the concentration of tezosentan. A recovery of 102.2% was determined for 500 ng/ml of I.S. Tezosentan was stable in all stability tests performed with no significant change in concentration.

Chromatograms of a blank human plasma sample, a quality control sample spiked with 1 ng/ml tezosentan and 500 ng/ml I.S., and a plasma sample obtained from a healthy volunteer 30 min after termination of an infusion of tezosentan 50 mg/h for 1 h are shown in Fig. 2. No interfering peaks were observed, demonstrating the selectivity of the method.

Table 1
Inter-assay imprecision and inaccuracy of quality control samples in human plasma

Amount added (ng/ml)	Amount found (ng/ml)	C.V. (%)	Inaccuracy (%)	n
Intra-day precision				
1.00	1.09±0.08	7.8	9.0	6
3.00	3.14±0.30	9.6	4.8	6
60.0	66.1±1.87	2.8	10.1	6
420	450±7.8	1.7	7.2	6
Inter-day precision				
1.00	1.08±0.07	6.5	8.1	4
3.00	3.07±0.06	2.0	2.4	4
60.0	67.2±0.8	1.1	12.0	4
420	462±11.9	2.6	9.9	4

4. Discussion and conclusion

A sensitive LC–MS–MS method was developed for the determination of tezosentan in human plasma. Accuracy and precision of the method were such that they fall within the criteria set for use in pharmacokinetic studies and in clinical trials with tezosentan [10]. No interfering peaks were observed, but gradient elution and column switching were needed in order to avoid ionization suppression.

The current method differs in several aspects from that reported for bosentan [8]. Most notably, the mobile phase does not contain ammonia, which was added to avoid carry-over in the bosentan method. Secondly, the analytical column was considerably shorter because no metabolites needed to be measured [9] as was the case for bosentan. The latter enabled a reduction of the run time to 6.5 min in the present method from 10 min for bosentan. The reported method has been applied in several phase I and II studies. It was shown that, in healthy subjects and patients, the plasma concentrations of tezosentan declined in a biphasic manner [6,7,11]. A representative plasma concentration–time curve obtained upon infusion of 50 mg/h of tezosentan for 1 h in a healthy subject is shown in Fig. 3.

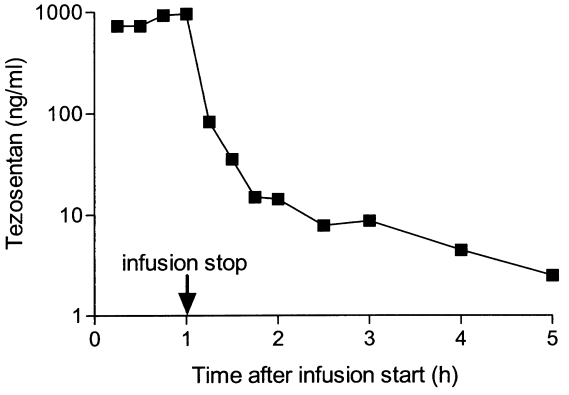


Fig. 3. Individual plasma concentration–time profile in a healthy subject receiving intravenous tezosentan as an infusion of 50 mg/h for 1 h. Samples with tezosentan concentrations greater than 512 ng/ml were diluted with plasma and re-assayed. Concentrations of tezosentan declined rapidly after infusion ceased and after 5 h were below the defined LOQ.

In conclusion, the described method may prove to be useful in pharmacokinetic studies of tezosentan and/or for therapeutic monitoring of this drug.

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