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Short communication

Detection of ketorolac enantiomers in human plasma using enantioselective liquid chromatography

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Abstract

A high-performance liquid chromatographic method for the determination of the enantiomers of ketorolac in human plasma has been developed. Plasma samples containing ketorolac were acidified and extracted into diethyl ether. The ethereal extract was evaporated to dryness and the residue reconstituted in mobile phase before injection onto a Chiral-AGP column. The mobile phase was 2-propanol–20 m*M* potassium dihydrogenphosphate buffered to pH 7 (0.5:99.5, v/v). Detection was by ultraviolet absorbance at 320 nm. The detection limit was 5 ng/ml for each enantiomer. The method has been applied to determine the concentration of ketorolac enantiomers during an infusion of the racemic drug and has proven to be rapid and sensitive.

1. Introduction

Ketorolac, 5-benzoyl-1,2,3*H*-pyrrole-1-carboxylic acid (Fig. 1) is a potent non-steroidal anti-inflammatory drug (NSAID) used for the short-term management of moderate to severe pain [1]. The *S*-enantiomer of ketorolac has been shown to be a significantly more potent analgesic than the *R*-enantiomer in animal studies [2]. A previously published chiral HPLC analysis [3] used derivatization with thionyl chloride–*S*-1-phenylethylamine to separate the diastereomers of ketorolac. This report describes the development and application of a rapid, simple and sensitive HPLC method for the quantitation of ketorolac enantiomers in human plasma, using an extraction method and chiral stationary phase similar to those described in a recent report on the separation of ketoprofen, ibuprofen and fenoprofen enantiomers [4]. The method requires no derivatisation, minimal sample preparation and results in run times of less than 10 min.

2. Experimental

2.1. Reagents and solvents

2-Propanol was liquid chromatography grade from Waters (Millipore, Lane Cove, Australia). Diethyl ether was nanograde from Mallinkrodt (Paris, KY, USA). Hydrochloric acid was ACS grade from Sigma (St. Louis, MO, USA). Potassium dihydrogenphosphate (99%) was from Sigma. Ketorolac tromethamine (Syntex, Palo

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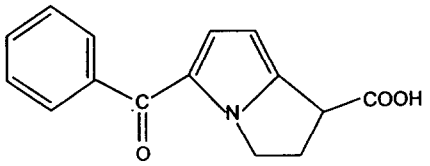

Fig. 1. Chemical structure of ketorolac.

Alto, CA, USA) and naproxen (Sigma) were analytical grade.

2.2. Chromatography

The HPLC system consisted of a WISP 712 autosampler, a Model 510 pump and a Model 481 absorbance detector, all from Waters (Millipore, Milford, MA, USA) interfaced to a PC running Maxima 820 software from Dynamic Solutions (Millipore). The column was a Chiral-AGP column (100 × 4.0 mm I.D., 5 μm) from Chromtech (Norsborg, Sweden). The mobile phase was 2-propanol–20 m*M* potassium dihydrogen phosphate buffered to pH 7 (0.5:99.5, v/v), running at a flow-rate of 0.8 ml/min. The column effluent was monitored at 320 nm. Under these conditions the retention times of *R*-ketorolac, *S*-ketorolac and naproxen were 3.5, 4.6 and 7.6 min, respectively.

2.3. Sample preparation

Plasma (500 μl), hydrochloric acid (200 μl, 2 *M*), methanolic naproxen (50 μl, 200 μg/ml) and diethyl ether (2 ml) were mixed in a sealable polypropylene centrifuge tube, vortex-mixed for 10 s and then centrifuged for 10 min at 1000 *g*. The ethereal phase was removed to a second polypropylene tube and evaporated to dryness under a stream of nitrogen. The residue was dissolved in 100 μl of mobile phase and a 10–100 μl aliquot was injected onto the column.

2.4. Calibration curve

Triplicate samples of spiked plasma were prepared at concentrations of 20, 10, 5, 2, 1, 0.5, 0.05 and 0.01 μg/ml of *R*,*S*-ketorolac for the determination of the calibration curve. Five samples of spiked plasma were prepared at 2 μg/ml of *R*,*S*-ketorolac for the determination of coefficients of variation for the enantiomers. Triplicate samples of spiked plasma were prepared at 20 and 10 ng/ml of *R*,*S*-ketorolac for the determination of the limit of detection.

2.5. Identification of the enantiomers

The order of elution of the enantiomers of ketorolac was established by personal communication with Syntex Laboratories [5] who reported that under the chromatographic conditions used in the assay described herein *R*-ketorolac is eluted before *S*-ketorolac.

2.6. Application of the method

The study was approved by the Hospital Ethics Committee and informed consent was obtained from a healthy male patient undergoing major abdominal surgery. The patient received an intravenous infusion of racemic ketorolac (Toradol, Syntex) at 24 mg/h for the first 30 min, followed by 3 mg/h for the next three days. Venous blood samples were taken at 09:00 h for the three days following surgery. Blood samples were centrifuged for 10 min at 4000 *g* and the plasma stored at −20°C until analysis.

3. Results and discussion

Typical chromatograms of blank human plasma and human plasma spiked with *R*,*S*-ketorolac (1 μg/ml of each enantiomer) are shown in Fig. 2. Baseline separation of the enantiomers is evident, with capacity factors (k') of 1.4, 2.2, and 4.2 for *R*-ketorolac, *S*-ketorolac and naproxen. The enantioselectivity factor (α) and enantioselective resolution factor (R_s) were 1.6 and 2.5, respectively. Linear regression of peak-area ratio versus concentration over the range 10–0.005 μg/ml of each of the enantiomers of ketorolac yielded coefficients of determination of 0.995 and 0.998 for *R*-ketorolac and *S*-ketorolac, respectively. The coefficients of variation at 1 μg/ml were found to be 2.3 and 3.5% for *R*-ketorolac and *S*-ketorolac, respectively. The

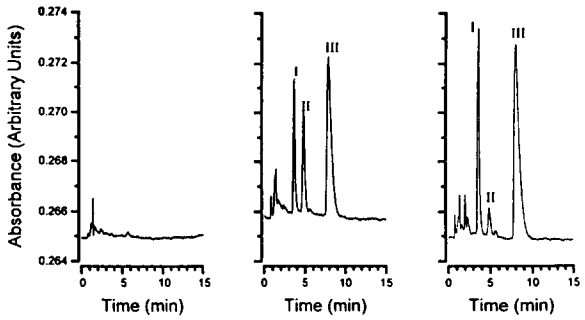

Fig. 2. Chromatograms of blank human plasma (left); blank human plasma spiked with racemic ketorolac to 1 μg/ml for each enantiomer and the internal standard, naproxen (10 μg/ml) (centre); and plasma obtained after 24 h of continuous infusion with racemic ketorolac (1.17 μg/ml *R*-ketorolac and 0.152 μg/ml *S*-ketorolac) (right). Peaks: I = *R*-ketorolac, II = *S*-ketorolac and III = naproxen. Injection volume: 20 μl.

limit of detection was 5 ng/ml for each enantiomer (with a signal-to-noise ratio of 5:1).

A sample chromatogram obtained from the analysis of human plasma samples taken daily during a 3-day infusion of racemic ketorolac is shown in Fig. 2. The plasma levels of the enantiomers on each day are summarised in Table 1. The levels of *R*-ketorolac are seven to ten times higher than the levels of *S*-ketorolac during the infusion. This figure compares with a five-fold difference in the concentration of *R*-ketorolac over *S*-ketorolac reported by Hayball et al. [3] eight hours after a volunteer received a 30-mg intramuscular dose of racemic ketorolac.

Table 1
Concentrations of ketorolac enantiomers during a continuous infusion of racemic ketorolac (Toradol, Syntex)

Day	*R*-Ketorolac (μg/ml)	*S*-Ketorolac (μg/ml)
1	1.17	0.152
2	0.94	0.090
3	1.15	0.118

Note added in proof

After acceptance of this manuscript another method was published for the direct resolution of the enantiomers of ketorolac and *p*-hydroxy-ketorolac using a human serum albumin-based chiral stationary phase [6].

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