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Short communication

Determination of ketorolac enantiomers in plasma using enantioselective liquid chromatography on an α_1-acid glycoprotein chiral stationary phase and ultraviolet detection

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Abstract

A chirally selective high-performance liquid chromatographic assay was developed to measure the *R*(+) and *S*(−) enantiomers of ketorolac in plasma for pharmacokinetic studies. Naproxen sodium [*S*(+) enantiomer] (10 μg) was used as an internal standard. Plasma samples (0.5 ml) were acidified (50 μl of 4 *M* H_3PO_4 to pH 1.5), extracted into 0.4 ml of 10% pentan-2-ol in hexane and back-extracted into 0.15 ml of base (20 m*M* NaOH pH to 7–8), of which samples (5 μl) were chromatographed on a 100 × 4 mm I.D. column packed with an HPLC chiral stationary phase based upon immobilized α_1-acid glycoprotein (Chiral AGP-CSP) with 4% propan-2-ol in 0.1 *M* NaH_2PO_4 pH 5.5, at 0.9 ml/min. Detection was at 325 nm and run time was 10 min. Retention times of *R*- and *S*-ketorolac and of *S*(+)-naproxen were 3.3, 4.8 and 6.4 min, respectively. The metabolite *p*-hydroxyketorolac was not resolved enantiomerically and had a retention time of 2.2 min. The assay was linear over the range 0.5–10 mg/l, with precisions <5% C.V. Good separations ($\alpha > 1.35$) and resolutions ($R_s > 3.23$) between peaks were achieved. The sensitivity could be extended to 35 μg/l with less precision by increasing the injection volume to 100 μl.

1. Introduction

Ketorolac trometamol (Toradol) (Fig. 1) is a nonsteroidal anti-inflammatory agent widely used in acute pain management [1]. It is administered as a racemate, the *S*(−) enantiomer being active analgetically [2]. The analysis of ketorolac in blood and/or plasma is relevant clinically for understanding its kinetics and thereby facilitating the development of dosing regimens and to perform other pharmacological investigations, for example, using drug combinations of ketorolac with opioids for providing increased analgesia without increasing the potentially toxic ventilatory depression of opioids, a side effect absent in ketorolac [3,4].

Various methods have been described for the measurement of ketorolac without differentiating between the enantiomers [5–9] and an enantioselective HPLC assay based on the formation of phenyethylamine diastereomers has been reported [10]. The method described here allows

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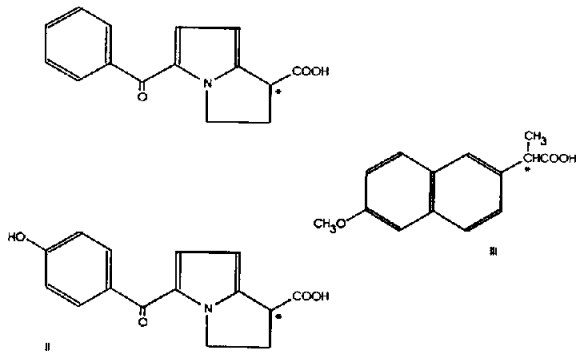

Fig. 1. Structures of ketorolac (I), *p*-hydroxyketorolac (II) and naproxen (III); * denotes the chiral centre.

direct measurement of the ketorolac enantiomers without the need for such derivatization.

2. Experimental

2.1. Reagents

Ketorolac trometamol and *S*(+)-naproxen sodium working standards (Fig. 1) were from Syntex Australia (North Sydney, N.S.W., Australia). Pure enantiomers of ketorolac and racemic *p*-hydroxyketorolac (Fig. 1) were from Dr P. Hayball (Pharmacy Department, Repatriation General Hospital, Daw Park, S.A., Australia). Propan-2-ol (HPLC "Unichrom" grade), di-sodium hydrogen phosphate and sodium dihydrogen phosphate (both analytical grade) were from Ajax Chemicals (Auburn, N.S.W., Australia). Pentan-2-ol (laboratory grade), orthophosphoric acid (analytical grade) and sodium hydroxide (analytical volume solution), were from BDH Chemicals (Kilsyth, Vic., Australia). Hexane (as mixed isomers with 95% *n*-hexane, "Nanograde") was from Mallinckrodt Speciality Chemicals (Paris, KY, USA). Water was purified by a Millipore Milli-Q system (Lane Cove, N.S.W., Australia). Helium (ultrapure) was from Commonwealth Industrial Gases (Parramatta, N.S.W., Australia).

2.2. Equipment

For chromatography the 600-MS system controller with pump, the 700 satellite WISP sample injector, the 911 photodiode array (PDA) detector and the 5200 printer/plotter were from the Waters division of Millipore. The system was controlled by a 386 personal computer. The 100 × 4 mm I.D. Chiral AGP-CSP column and the 10 × 3 mm I.D. diol guard column were from ChromTech AB (Norsborg, Sweden). A 4 × 6 mm I.D. Waters Guard-Pak C_{18} pre-column was placed between the pump and injector.

2.3. Standards

Ketorolac trometamol used as standard was stored as stock at 2.95 g/l [1 g/l each of *R*(+)- and *S*(−)-ketorolac as acids] in water for up to 2 months at 4°C in 15-ml polypropylene tubes. Working solutions of 200, 100, 40, 20, 10 and 4 mg/l, which could be stored for up to 2 months at 4°C, were made by diluting 1 ml of stock in 4 ml of water and serially diluting 2 ml of this solution in 2, 3, 2, 2 and 3 ml of water. For standard calibration and yield computation 50-μl volumes of these ketorolac standards and water blank were added to 0.95 ml blood or plasma samples free of ketorolac to correspond to 10, 5, 2, 1, 0.5, 0.2 and 0 mg/l.

Naproxen sodium [*S*(+) enantiomer] was used as internal standard and stored as stock at 1 g/l in water for up to 2 months at 4°C in 15-ml polypropylene tubes. A working solution of 0.2 g/l was made by diluting 1.1 ml of stock in 4.4 ml of water for every 100 samples. This solution could be stored for up to 2 months at 4°C. Aliquots of 50 μl (10 μg) of this working solution were added to each 0.5-ml plasma sample (17 mg/l naproxen as acid).

Ketorolac trometamol for injection consisted of 90 mg in 3 ml aqueous vehicle containing 300 mg ethanol and 13 mg sodium chloride.

2.4. Extraction

Fresh blood samples of ca. 1 ml were centrifuged in 1.5-ml Eppendorf tubes at 5000 rpm (ca. 1000 *g*) for 10 min, and 0.5-ml aliquots of plasma were transferred to 1.5-ml Eppendorf tubes and could be stored at −20°C for up to 4 weeks. The aliquots of plasma had 50 μl of 4 *M* H_3PO_4 (reducing the pH to 1.5), 50 μl of

naproxen internal standard, and 0.4 ml of 10% pentan-2-ol in hexane added, and were then mixed 4 times at 1000 rpm for 30 s, centrifuged at 5000 rpm for 5 min and frozen in solid CO_2 for 5 min. The (upper) organic phase was decanted into 1.5-ml Eppendorf tubes containing 0.15 ml of 20 m*M* NaOH (raising the pH to 7–8), mixed 4 times at 1200 rpm for 30 s, centrifuged at 3000 rpm for 6 min and frozen. The (upper) organic phase was discarded, the tube openings blotted immediately on clean filter paper and the samples allowed to warm for 5 min to >0°C before being mixed at 1200 rpm for 30 s and centrifuged at 5000 rpm for 5 min, whereupon 0.15-ml aliquots were transferred into 0.25-ml insert vials, which were placed in HPLC vials for analysis.

2.5. Chromatography

Samples (5–100 μl) were chromatographed on a 100 × 4 mm I.D. Chiral AGP-CSP column protected by a diol guard column with 4% propan-2-ol in 0.1 *M* NaH_2PO_4 (pH 5.5) at 0.9 ml/min and at ambient temperature (ca. 23°C). A C_{18} pre-column was placed between the pump and injector to scavenge organics from the mobile phase, which was recycled and sparged with helium at 1 ml/min. Detection was by UV-absorption at 325 nm × 10 nm slit width. The run time was 10 min. Up to 96 samples could be assayed in an unattended overnight run. All the columns could be cleaned with 25% propan-2-ol in water as required.

2.6. Calculations

Calibration was done by the PDA software and used the internal standard method with 6 points derived from peak areas corresponding to 10, 5, 2, 1, 0.5 and 0.2 mg/l of each ketorolac enantiomer. Linearity was calculated from these points by linear regression. Variability was expressed as percentages of the coefficients of variation (%C.V.s) from values of 5 replicates of each ketorolac enantiomer at 5.0 and 0.5 mg/l (5-μl injections) and 70 and 35 μg/l (100-μl injections). Extraction yields were expressed as percentages derived from peak areas of 5 replicates of each ketorolac enantiomer at 5.0 and 0.5 mg/l and naproxen at 17 mg/l compared to corresponding unextracted standards.

2.7. Example of application of the method

An adult merino ewe was administered 90 mg of racemic ketorolac trometamol intravenously over 10 s. Arterial blood samples of 1 ml were then taken periodically (at 1, 3, 5, 10, 20, 45, 60, and 90 min) via a chronically placed in-dwelling catheter and assayed as described.

3. Results

The retention times of *R*- and *S*-ketorolac and of naproxen were 3.3, 4.8 and 6.4 min, and their capacity factors (k') were 2.83, 4.09 and 5.52, respectively. The metabolite *p*-hydroxyketorolac was well separated from the parent compound, but was not resolved enantiomerically. Its retention time was 2.2 min, and capacity factor 1.87. Separate injections of the pure enantiomers of ketorolac were made to confirm their identity. Fig. 2 depicts a blank extracted sample with and without 2.5 mg/l ketorolac added; a sample containing injected *R*-ketorolac (2.05 mg/l) and *S*-ketorolac (1.13 mg/l), each with 17 mg/l naproxen; and the pure standards (1 mg/l) and naproxen (17 mg/l). Separations (α) and resolutions (R_s) between successive peaks were 1.52 and 3.70, 1.45 and 3.79, and 1.35 and 3.23, respectively.

The linearity of the standard range (0–10 mg/l) was shown by correlation coefficients >0.998 for *R*-ketorolac and >0.999 for *S*-ketorolac. The intercept was not significantly different from zero ($p = 0.51$ for *R*-ketorolac and 0.41 for *S*-ketorolac).

The standard method (using 5-μl injections) produced for *R*- and *S*-ketorolac, respectively, C.V.s in blood of 1.7 and 2.0% at 5 mg/l, and 6.1 and 2.6% at 0.5 mg/l, and in plasma 5.5 and 5.4% at 5 mg/l, and 5.3 and 5.4% at 0.5 mg/l. The extended method (using 100-μl injections) produced C.V.s of 4.1 and 2.7% at 70 μg/l and 32 and 17% at 35 μg/l.

The apparent yields for *R*- and *S*-ketorolac

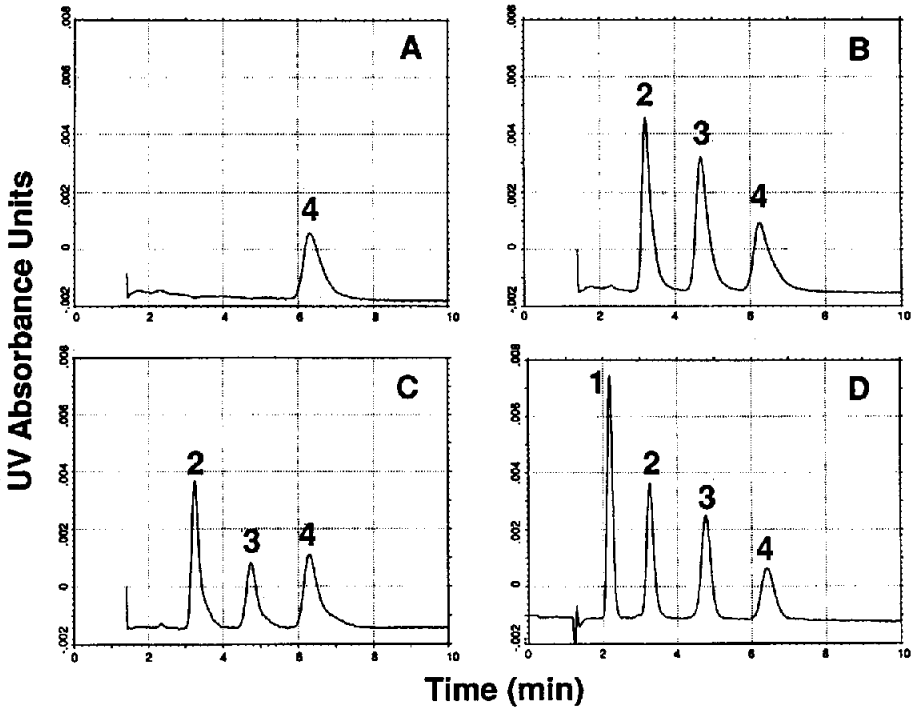

Fig. 2. Chromatograms of (A) extract of blank plasma from blood spiked with 17 mg/l of naproxen (4) as internal standard, (B) the same spiked with 2.5 mg/l of *R*-ketorolac (2) and 2.5 mg/l of *S*-ketorolac (3), (C) extract of a plasma sample containing 2.05 mg/l of *R*-ketorolac (2) and 1.13 mg/l of *S*-ketorolac (3), obtained 90 min after injection of 90 mg of racemic ketorolac trometamol bolus i.v., (D) pure standards 1 mg/l each of *p*-hydroxyketorolac enantiomers unresolved (1), 1 mg/l of *R*-ketorolac (2) and 1 mg/l of *S*-ketorolac (3) and 14 mg/l of naproxen (4). Their retention times were 2.2, 3.3, 4.8 and 6.4 min, respectively. Samples, 5 μl (panels A–C) and 10 μl (D), were chromatographed on a 100 × 4 mm I.D. Chiral AGP-CSP column with 4% propan-2-ol in 0.1 *M* NaH_2PO_4 (pH 5.5) at 0.9 ml/min and at ca. 23°C, detection by UV-absorption at 325 nm.

respectively in blood were 69 and 72% at 5 mg/l, and 72 and 73% at 0.5 mg/l, and in plasma were 59 and 59% at 5 mg/l, and 60 and 59% at 0.5 mg/l. The yield for naproxen was 75% at 17 mg/l. These results are shown in Table 1. The higher yields from blood could indicate incomplete equilibrium between plasma and blood cells after addition of ketorolac and mixing for 10 min.

The ketorolac blood concentration–time course after intravenous administration in the example is shown in Fig. 3.

4. Discussion

The present method compares well with previously published methods [5–10] with regard to sensitivity and precision, and also is simpler to perform. It has the advantage of measuring the enantiomers directly, with no more effort than the racemate. Blood concentrations can be determined by respectively spiking either with the standard curve, prior to centrifugation and harvesting plasma for assay. The organic extraction removes the ketorolac from plasma, in which it is for approx. 99% bound to protein in humans [6]. Previous methods [6,10] have used ethyl acetate as extractant; however, having found that this tended to lower the pH of the back extractant, pentan-2-ol was used instead, thereby especially improving the yield of naproxen. Naproxen levels in solution progressively decreased after successive transfers through glass (but not polypropylene) tubes, indicating that naproxen binds to glass, which could reduce its recovery.

Table 1
Extraction recovery and assay precision

Concentration spiked (as acid) (mg/l)		Injection volume (μl)	Yield (%)	
			Blood	Plasma
Standard method				
5	*R*-Ketorolac	5	69.1 (1.7)	58.5 (5.5)
	S-Ketorolac	5	71.9 (2.0)	59.1 (5.4)
0.5	*R*-Ketorolac	5	71.5 (6.1)	60.0 (5.3)
	S-Ketorolac	5	72.7 (2.6)	59.5 (5.4)
17	Naproxen	5	74.8	
Extended method				
0.07	*R*-Ketorolac	100	(4.1)	
	S-Ketorolac	100	(2.7)	
0.035	*R*-Ketorolac	100	(32.4)	
	S-Ketorolac	100	(17.1)	

This assay is suitable for routine analysis of blood or plasma and for determination of the pharmacokinetics of ketorolac enantiomers. Blood concentrations were determined via assay of plasma since blood tended to coagulate unless diluted, thereby effectively reducing assay sensitivity. The assay can be used clinically and experimentally, for the investigation of various body fluids or tissue homogenates. For example, ketorolac concentrations (typically 250 mg/l) have been measured in urine by direct injection of 2-μl aliquots of a 1/50 dilution in water, using the same chromatographic conditions. The analysis of ketorolac is considered to be a valuable tool in the development of effective pain management strategies using this agent.

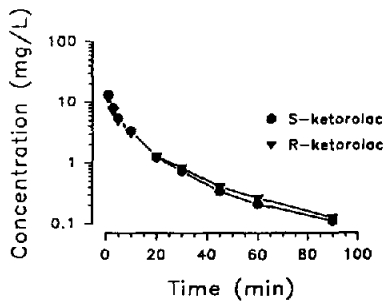

Fig. 3. Example of an application of the method: time–concentration profile of ketorolac enantiomers in blood after intravenous injection of 90 mg of ketorolac trometamol as a bolus to an adult ewe.

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References

[1] M.M.-T. Buckley and R.N. Brogden, *Drugs*, 39 (1990) 86.
[2] A. Guzman, F. Yuste, R.A. Toscano, J.M. Young, A.R. Van Horn and J.M. Muchowski, *J. Med. Chem.*, 29 (1986) 589.
[3] L.J.C. Brandon Bravo, H. Mattie, J. Spierdijk, J.G. Bovill and A.G.L. Burm, *Eur. J. Clin. Pharmacol.*, 35 (1988) 491.
[4] G.F. Rich, R. Schacterle, J.C. Moscicki, C.A. DiFazio and J.M. Adams, *Anaesth. Analg.*, 75 (1992) 99.
[5] D. Jung, E.J. Mroszczak, A. Wu, T.L. Ling, H. Sevelius and L. Bynum, *Pharmaceut. Res.*, 6 (1989) 62.
[6] N.S. Jallad, D.C. Garg, J.J. Martinez, E.J. Mroszczak and D.J. Weidler, *J. Clin. Pharmacol.*, 30 (1990) 76.

[7] A.T. Wu and I.J. Massey, *J. Chromatogr.*, 534 (1990) 241.
[8] K.T. Olkkola and E.-L.Maunuksela, *Br. J. Clin. Pharmac.*, 31 (1991) 182.
[9] R.S. Chaudhary, S.S. Gangwal, K.C. Jindal and S. Khanna, *J. Chromatogr.*, 614 (1993) 180.
[10] P.J. Hayball, J.G. Tamblyn, Y. Holden and J. Wrobel, *Chirality*, 4 (1992) 1.