Journal of Chromatography, 382 (1986) 382—388
Biomedical Applications
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROMBIO. 3282

Note

High-performance liquid chromatographic analysis of piroxicam and its major metabolite 5′-hydroxypiroxicam in human plasma and urine

C. JANE RICHARDSON and SHARON G. ROSS

College of Pharmacy, University of Saskatchewan, Saskatoon, Saskatchewan S7N 0W0 (Canada)

KEN L. BLOCKA

College of Medicine, University of Saskatchewan, Saskatoon, Saskatchewan S7N 0W0 (Canada)

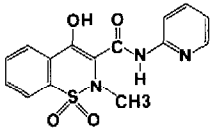
and

ROGER K. VERBEECK*

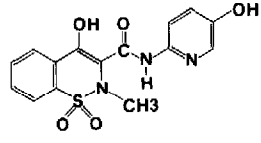
College of Pharmacy, University of Saskatchewan, Saskatoon, Saskatchewan S7N 0W0 (Canada)

(First received April 9th, 1986, revised manuscript received June 10th, 1986)

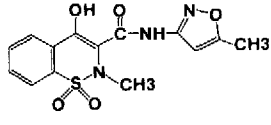
Piroxicam (Fig. 1) is a non-steroidal anti-inflammatory drug belonging to a novel class of compounds called the oxicams [1]. It is widely used in the treatment of patients with rheumatological disorders [2]. The pharmacology,



PIROXICAM



5′ - HYDROXYPIROXICAM



ISOXICAM

Fig. 1. Chemical structures of piroxicam [4-hydroxy-2-methyl-N-2-pyridynyl-2-H-1,2-benzothiazine-3-carboxamide 1,1-dioxide], isoxicam [4-hydroxy-2-methyl-N-2-(5-methyl-3-isoxazolyl)-2H-1,2-benzothiazine-3-carboxamide 1,1-dioxide] and 5′-hydroxypiroxicam.

0378-4347/86/$03.50

pharmacokinetics and clinical efficacy of piroxicam have recently been summarized [2].

In the earlier pharmacokinetic studies of piroxicam in man, plasma levels were measured by spectrophotometric [3, 4] or fluorimetric wet chemistry quantitation [4, 5]. These methods lack sensitivity and selectivity and are now replaced by a number of high-performance liquid chromatographic (HPLC) methods with UV detection [6—10]. However, in all these methods little attention was paid to 5′-hydroxypiroxicam, the major metabolite of piroxicam in man [11]. Recently, Tsai et al. [12] published an HPLC method for the simultaneous quantitation of piroxicam and 5′-hydroxypiroxicam in plasma and urine of rabbits. The sensitivity limit for both compounds was 0.5 μg/ml.

The present report describes the simultaneous quantitation of piroxicam and 5′-hydroxypiroxicam in human plasma and urine. Sensitivity limits for both compounds are 50 ng/ml in plasma and 100 ng/ml in urine. The applicability of the methods has been assessed by analysis of plasma and urine samples from volunteers following administration of multiple doses of piroxicam (20 mg/day).

EXPERIMENTAL

Chemicals

Piroxicam and 5′-hydroxypiroxicam were obtained from Pfizer (Groton, CT, U.S.A.). Isoxicam (internal standard) was a gift from Warner Lambert/Parke Davis (Ann Arbor, MI, U.S.A.). The structures of these compounds are shown in Fig. 1. Tetrahydrofuran and acetonitrile were both HPLC-grade solvents (BDH Chemicals, Canada). Octyl sodium sulfonate was purchased from Pfaltz and Bauer (Waterbury, CT, U.S.A.). Bovine liver β-glucuronidase (Type B-1) and tris(hydroxymethyl)aminomethane were purchased from Sigma (St. Louis, MO, U.S.A.). Diethyl ether (anhydrous, BDH Chemicals) was always distilled in glass just before use. All other chemicals were of analytical grade and obtained from Fisher Scientific, Canada.

Extraction of plasma

To 0.5 ml of plasma in a 12.0-ml glass extraction tube (Kimax Owens-Illinois, Toledo, OH, U.S.A.) were added: 1 ml of 0.01 *M* sodium hydroxide (containing piroxicam and 5′-hydroxypiroxicam in varying concentrations when preparing standards), 0.1 ml of internal standard solution (0.1 mg isoxicam per ml of 0.1 *M* sodium hydroxide), 0.25 ml of 1 *M* hydrochloric acid and 5 ml of freshly distilled diethyl ether. The stoppered tubes were shaken mechanically for 5 min using an Ika-Vibrax-VXR shaker (Ika-Werk, Staufen, F.R.G.) and centrifuged at 1150 *g* for 5 min at 4°C (AccuSpin-FR, Beckman Instruments, Palo Alto, CA, U.S.A.). The ether phase was transferred to a clean tube and evaporated to dryness in a waterbath (35°C) under a gentle stream of nitrogen (N-Evap, Organomation Assoc., South Berlin, MA, U.S.A.). The residue was reconstituted in 250 μl of 0.05 *M* tris(hydroxymethyl)aminomethane immediately prior to injection (50 μl) onto the column.

Extraction of hydrolysis of urine

The extraction procedure for unconjugated piroxicam and 5′-hydroxypiroxicam in urine was identical to the one used in plasma. For measurement of 5′-hydroxypiroxicam glucuronide, urine adjusted to pH 5.0 with an equal volume of 0.1 *M* acetate buffer was incubated overnight in the presence of 500 I.U. bovine liver β-glucuronidase. Internal standard was added and after acidification the incubation mixture was extracted with diethyl ether as described above. Residues were reconstituted in 100 μl of 0.05 *M* tris(hydroxymethyl)aminomethane immediately prior to injection (10 μl) onto the column.

Reversed-phase chromatography

Instrumentation included a Rheodyne 7125 injector (Rheodyne, Cotati, CA, U.S.A.), a Waters M-45 pump (Waters Assoc., Milford, MA, U.S.A.), a fixed-wavelength UV detector (Waters Model 44) and a chart recorder (Linear Instruments, Irvine, CA, U.S.A.). For simultaneous quantitation of piroxicam and 5′-hydroxypiroxicam in plasma a μBondapak CN column (15 cm × 3.9 mm, 10 μm particle size, Waters Assoc.) was used. The mobile phase consisted of 50 m*M* NaH_2PO_4 in acetonitrile—water (25:75) with a final pH of 3.2. The flow-rate was 1.5 ml/min and the eluate was monitored at 365 nm. For the urine assay an aliquot of the extraction residue was injected onto a μBondapak C_{18} column (30 cm × 3.9 mm, 10 μm particle size, Waters Assoc.). The mobile phase consisted of 5 m*M* sodium sulfonate buffer—tetrahydrofuran—glacial acetic acid (54:45:1) and was pumped through the column at room temperature. The flow-rate was set at 1.5 ml/min. The eluate was monitored at 365 nm.

Pharmacokinetic study

Six volunteers ingested a 20-mg piroxicam capsule every morning for fifteen consecutive days. Blood samples were periodically withdrawn during drug administration and following ingestion of the last dose. Blood, collected in a heparinized evacuated tube (Venoject, Terumo Medical Corp., Elkton, MD, U.S.A.), was immediately centrifuged and plasma was stored at −20°C until analyzed. Blank (predose) and 24-h urine samples (steady state) were collected and aliquots stored at −20°C. A detailed description of the results of this study will be published elsewhere [13].

RESULTS

Extraction recoveries

Recoveries of piroxicam and 5′-hydroxypiroxicam added to plasma and urine were high and ranged between approximately 80 and 90% (see Tables I and II). Recovery of the internal standard was between 88 and 92%.

Linearity

A straight line was obtained by plotting peak-height ratios of piroxicam or 5′-hydroxypiroxicam over internal standard versus concentration in plasma or urine. For plasma, the relationship was linear in the range 50—5000 ng/ml for piroxicam and 50—1000 ng/ml for 5′-hydroxypiroxicam. For urine, linear standard curves were obtained between 100 and 5000 ng/ml for both piroxi-

TABLE I

EXTRACTION RECOVERIES AND COEFFICIENTS OF VARIATION IN REPLICATE STANDARDS (n=4) EXTRACTED FROM HUMAN PLASMA

Concentration (ng/ml)	Recovery (%)		Coefficient of variation (%)	
	Piroxicam	5′-Hydroxypiroxicam	Piroxicam	5′-Hydroxypiroxicam
50	86.1	92.7	1.3	1 0
100	81 5	90.8	2.3	3.3
250	85.4	88.3	1.2	0.8
500	88.6	91.6	1.9	2.9
1000	86.5	84.5	0.5	2.6
2500	84.0	—	1.4	—
5000	84.6	—	0.7	—

TABLE II

EXTRACTION RECOVERIES AND COEFFICIENTS OF VARIATION IN REPLICATE STANDARDS (n=4) EXTRACTED FROM HUMAN URINE

Concentration (ng/ml)	Recovery (%)		Coefficient of variation (%)	
	Piroxicam	5′-Hydroxypiroxicam	Piroxicam	5′-Hydroxypiroxicam
100	91.5	84.0	2.6	6.0
250	89.2	86.3	3.5	3.1
1000	82.0	83.7	3.3	3.3
2500	82 6	85.2	4.0	1.7
5000	79.8	81.5	3.3	1.3

cam and its major metabolite. Correlation coefficients of the standard curves were typically higher than 0.999 with intercepts not significantly different from zero.

Precision and accuracy

Bulk control standards were prepared by adding known amounts of piroxicam and 5′-hydroxypiroxicam to blank plasma and urine. Four aliquots of each control standard were analyzed and the coefficients of variation are summarized in Tables I and II. For all concentration levels studied the coefficient of variation was 6.0% or smaller. The average (n=8) back-calculated concentrations of piroxicam and 5′-hydroxypiroxicam in plasma and urine were within 5% of the seeded values at concentrations of 250 and 1000 ng/ml.

Selectivity

No endogenous interfering peaks were visible in blank plasma or urine at the retention times of piroxicam, 5′-hydroxypiroxicam and isoxicam (Figs. 2 and 3). Incubation of urine samples overnight at 37°C in the presence of β-glucuronidase did not result in the occurrence of any interfering substances.

Sensitivity

The lower limits of sensitivity for piroxicam and its 5′-hydroxy metabolite were 50 and 100 ng/ml in plasma and urine, respectively.

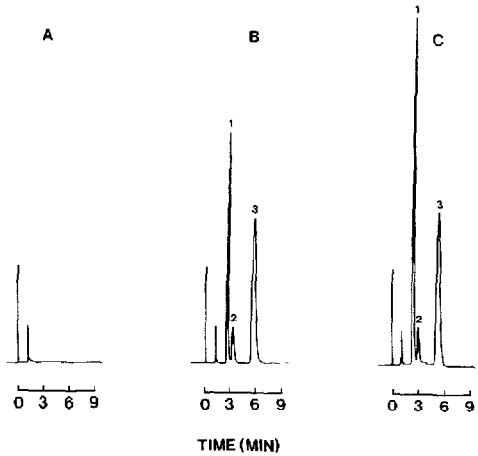


Fig 2. Chromatograms of plasma extracts for (A) blank plasma, (B) blank plasma spiked with 1000 ng/ml piroxicam (1), 500 ng/ml 5′-hydroxypiroxicam (2) and internal standard (3), and (C) plasma sample (0 5 ml extracted) from a volunteer at steady state (2845 ng/ml piroxicam and 935 ng/ml 5′-hydroxypiroxicam).

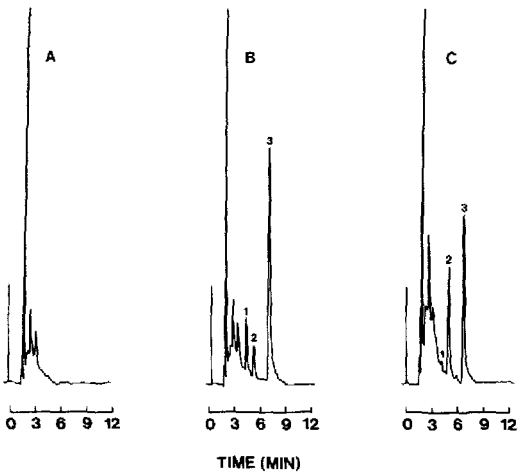


Fig. 3. Chromatograms of urine extracts for (A) blank urine, (B) blank urine spiked with 250 ng/ml piroxicam (1), 250 ng/ml 5′-hydroxypiroxicam (2) and internal standard (3), and (C) urine sample (1 ml extracted) obtained from a volunteer at steady state (1190 ng/ml).

Pharmacokinetic study

Fig. 4 shows the semilogarithmic plasma concentration—time profiles for piroxicam and 5′-hydroxypiroxicam during administration and following discontinuation of 20 mg piroxicam once daily for fourteen days to a young, healthy volunteer. Steady-state plasma concentrations of the hydroxy metabolite are approximately 20% of those of piroxicam. On average 25.2 ± 3.4% (n=6) of the dose was recovered in urine at steady state as 5′-hydroxypiroxicam. Approximately 65% of this total recovery of 5′-hydroxy metabolite was in the form of the glucuronide conjugate. No measurable concentrations of piroxicam were found in urine.

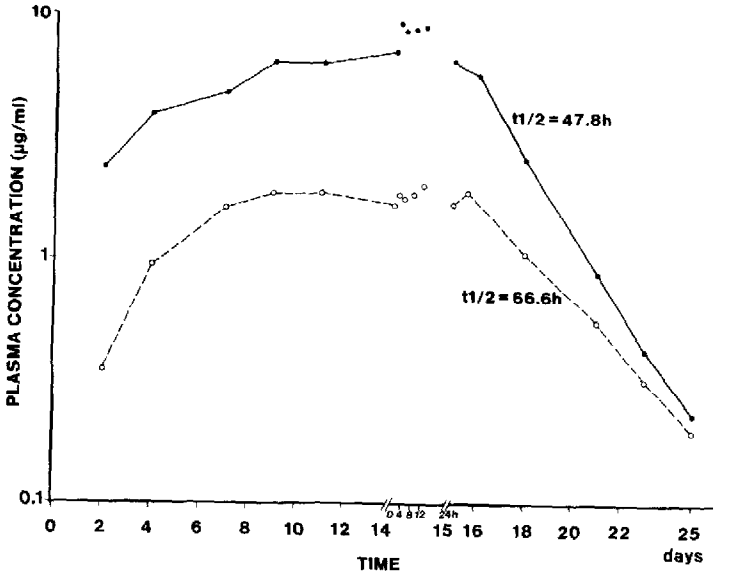


Fig. 4. Semi-logarithmic plasma concentration—time profiles for piroxicam (•) and 5′-hydroxypiroxicam (○) in a young, healthy volunteer during administration and following discontinuation of 20 mg piroxicam once a day.

DISCUSSION

This assay provides a rapid, sensitive and reproducible method for the simultaneous quantitation of piroxicam and its major metabolite (5′-hydroxypiroxicam) in human plasma or urine. The plasma assay is based on a previously published method [6], modified to obtain a baseline separation between piroxicam and its major metabolite. For analysis of urine, however, a different technique had to be used to separate piroxicam and 5′-hydroxypiroxicam from endogenous substances. The HPLC conditions we used for analysis of urine extracts were based on a method to quantitate isoxicam in plasma and urine [14].

The method as presented here has been routinely used in our laboratories for the analysis of piroxicam and 5′-hydroxypiroxicam in human plasma and urine in clinical studies [15, 16]. It has proven to be a simple procedure with a high degree of reproducibility. The method is sufficiently sensitive to monitor plasma levels of piroxicam for 216 h following administration of a single 20-mg piroxicam dose [15]. However, plasma levels of the metabolite are only measurable following multiple-dose administration of piroxicam. Following single-dose administration of piroxicam, 5′-hydroxypiroxicam plasma levels usually are very low and close to the sensitivity limit of the described method. In addition, urine concentrations of the metabolite can be reliably measured following multiple-dose piroxicam administration.

ACKNOWLEDGEMENTS

This work was supported by a grant from the Saskatchewan Health Research Board. The authors wish to thank Ms. J. Huck for typing the manuscript.

REFERENCES

1 H. Zinnes, J.C. Sircar, N. Lundo, M.L. Schwartz, A.C. Fabien, J. Shavel, Jr., C.F. Kasulanis, J.D. Genzer, C. Lutonski and G. DiPasquale, J. Med. Chem., 25 (1982) 12.
2 R.N. Brogden, R.C. Heel, T.M. Speight and G.S. Avery, Drugs, 28 (1984) 292.
3 E.H. Wiseman, Y.-H. Chang and J.G. Lombardino, Arzneim.-Forsch., 26 (1976) 1300.
4 D.C. Hobbs and T.M. Twomey, J. Clin. Pharmacol., 19 (1979) 270.
5 T. Ishizaki, T. Nomura and T. Abe, J. Pharmacokin. Biopharm., 7 (1979) 369.
6 T.M. Twomey, S.R. Bartolucci and D.C. Hobbs, J. Chromatogr., 183 (1980) 104.
7 P. Schiantarelli, D. Acerbi and G. Bovis, Arzneim.-Forsch., 31 (1981) 92.
8 J.-B. Fourtillan and D. Dubourg, Thérapie, 38 (1983) 163.
9 A.D. Fraser and J.F.L. Woodbury, Ther. Drug Monit., 5 (1983) 239.
10 J.S. Dixon, J.R. Lowe and D.B. Galloway, J. Chromatogr., 310 (1984) 455.
11 T.M. Twomey and D.C. Hobbs, Fed. Proc., Fed. Am. Soc. Exp. Biol., 37 (1978) 271.
12 Y.-H. Tsai, L.-R. Hsu and S.I. Naito, Int. J. Pharm., 24 (1985) 101.
13 C.J. Richardson, K.L.N. Blocka, S.G. Ross and R.K. Verbeeck, Eur. J. Clin. Pharmacol., in press.
14 A.C. Daftsios, E.L. Johnson, K.J. Keeley, C. Gryczko and V. Rawski, J. Chromatogr., 305 (1984) 145.
15 C.J. Richardson, K.L.N. Blocka, S.G. Ross and R.K. Verbeeck, Clin. Pharmacol. Ther., 37 (1985) 13.
16 R.K. Verbeeck, C.J. Richardson and K.L.N. Blocka, Clin. Pharmacol. Ther., 39 (1986) 224.