Journal of Chromatography, 616 (1993) 59–65
Biomedical Applications
Elsevier Science Publishers B.V., Amsterdam

CHROMBIO. 6839

Limited extent of stereochemical conversion of chiral non-steroidal anti-inflammatory drugs induced by derivatization methods employing ethyl chloroformate

Matthew R. Wright and Fakhreddin Jamali*

Faculty of Pharmacy and Pharmaceutical Sciences, University of Alberta, Edmonton, Alberta T6G 2N8 (Canada)

(First received January 5th, 1993; revised manuscript received March 11th, 1993)

ABSTRACT

A potential problem with chiral derivatization is the possibility of stereochemical conversion during the derivatization reaction. This possibility has been examined using the non-steroidal anti-inflammatory drugs ibuprofen, ketoprofen, etodolac and flurbiprofen. To avoid possible interference from stereochemical impurities, male Sprague–Dawley rats were dosed intraperitoneally with the *S* enantiomer (100 mg/kg) of each drug and the *R* enantiomer of etodolac. Blood samples were taken 4 h afterwards. The plasma samples were analyzed using published stereospecific methods involving chiral derivatization with ethyl chloroformate followed by either *R*-(+)-α-phenylethylamine or L-leucinamide. For all the drugs examined, the percentage of formation of the antipode was between 1.0 and 5.8%. *In vitro* studies of the *R* and *S* enantiomers demonstrate that the apparent extent of conversion is inversely related to the concentration of ethyl chloroformate present during the derivatization reaction for ibuprofen, ketoprofen and flurbiprofen but not for etodolac. However, both the *R* and *S* enantiomers appear to be inverted to the same extent in the presence of ethyl chloroformate. These results suggest that the degree of stereochemical conversion induced by these assay procedures is small and would not contribute significantly to analytical error in the absence of a large difference in concentrations of the enantiomers.

INTRODUCTION

The chromatographic resolution of enantiomers is based on the formation of diastereomers, either permanently (*e.g.* chiral derivatization methods) or transiently (*e.g.* chiral stationary phases, chiral mobile phase additives) [1]. A theoretical concern with methods that involve the use of chiral derivatization techniques is the possibility of stereochemical conversion of the analytes during the derivatization process [2,3]. This concern, however, has been largely speculative.

A large number of non-steroidal anti-inflammatory drugs (NSAIDs) are marketed as racemates and due to the great clinical importance of this pharmacological class there has been extensive investigation of these compounds (*e.g.* refs. 4–7) with a great deal of emphasis on the stereoselective behavior of the antipodes. As a consequence of this widespread interest in chiral NSAIDs a number of stereospecific analytical methods have been developed, principally using high-performance liquid chromatography (HPLC) following chiral derivatization (*e.g.* refs. 8–19). In addition to the theoretical concern of stereochemical conversion during the derivatization reaction [2,3], Ahn *et al.* [20] have suggested the stereochemical conversion of ibuprofen enantiomers during derivatization with ethyl chloroformate, a reagent employed in many of the assay procedures cited previously.

* Corresponding author.

0378-4347/93/$06.00

Determination of the existence of stereochemical conversion of chiral NSAIDs during derivatization is complicated by the contamination of stereochemically "pure" enantiomers by small amounts of the antipode. Therefore, direct determination of the extent of stereochemical conversion is difficult. In addition many 2-arylpropionate NSAIDs are bioinverted from the *R* to *S* antipode making *in vivo* studies with the *R* enantiomers difficult. However, there does not appear to be any significant metabolic inversion of the *S* enantiomers to the *R* enantiomers [21] nor does there appear to be bioinversion of *R*-etodolac [22].

The purpose of the present study was to examine the possibility of stereochemical conversion for NSAIDs when ethyl chloroformate was employed in the derivatization reaction prior to HPLC separation. In this study stereochemically "pure" *S* enantiomers were administered to rats to eliminate the possibility of metabolic *R* to *S* inversion and to allow clearance of any *R* enantiomer present as impurity in the dose of the *S* enantiomer. Subsequently, we examined the effect of changes in the concentration of ethyl chloroformate in the derivatization reaction on the observed stereochemical conversion of both *R* and *S* enantiomers.

EXPERIMENTAL

Chemicals

Pure *R* and *S* enantiomers of ibuprofen, ketoprofen, etodolac and flurbiprofen were the kind gifts of Sepracor (Marlborough, MA, USA), Bayer Leverkusen (Wuppertal, Germany), Wyeth-Ayerst (Philadelphia, PA, USA), and Organon Canada (Westhill, Canada), respectively. Ethyl chloroformate (BDH, Poole, UK), *R*-(+)-α-phenylethylamine (Sigma, St. Louis, MO, USA) and L-leucinamide (Sigma) were >99% pure.

Analytical procedures

Stereospecific ibuprofen, ketoprofen and flurbiprofen concentrations were determined using published reversed-phase HPLC methods [13,15,19]. The limits of quantitation of these methods were 0.25, 0.1 and 0.1 μg/ml for ibuprofen, ketoprofen and flurbiprofen, respectively [13,15,19]. Stereospecific etodolac concentrations were determined using a published normal-phase HPLC method with a limit of quantitation of 0.05 μg/ml [16].

All of the analytical methods used obtain enantiomeric separation via the formation of diastereomers following chiral derivatization. In each case the carboxylic acid moiety of the NSAID is activated to a carbonate using ethyl chloroformate prior to condensation with either *R*-(+)-α-phenylethylamine (ibuprofen, etodolac) or L-leucinamide (ketoprofen, flurbiprofen).

For each drug studied a calibration curve, over the appropriate concentration range, was prepared simultaneously with the plasma samples and used for the stereospecific quantitation.

Chromatographic conditions

The HPLC apparatus (Waters, Mississauga, Canada) consisted of a Model 590 pump, a Model 481 variable-wavelength detector, a 710B WISP autosampler and either a 10 cm × 4.6 mm I.D. C_{18} (for ibuprofen, ketoprofen and flurbiprofen) (Phenomenex, Torrance, CA, USA) or a 25 cm × 4.6 mm I.D. Partisil 5 Silica (Whatman, Clifton, NJ, USA) analytical column. All analyses were performed under ambient temperature. The recorder integrator was a Model 3390A (Hewlett-Packard, Palo Alto, CA, USA).

Mobile phases were acetonitrile–water–acetic acid–triethylamine (TEA) (46.5:53.5:0.1:0.03, v/v; flow-rate 1.6 ml/min) for ibuprofen, acetonitrile–0.067 *M* KH_2PO_4–TEA (35:65:0.02, v/v; flow-rate 1.0 ml/min) for ketoprofen, acetonitrile–0.067 *M* KH_2PO_4–TEA (35:65:0.02, v/v; flow-rate 1.0 ml/min) for flurbiprofen, and hexane–ethyl acetate–isopropanol (85:15:0.2, v/v; flow-rate 2.0 ml/min) for etodolac [13,15,16,19].

The detection wavelengths were 225 nm (ibuprofen), 250 nm (flurbiprofen) and 280 nm (ketoprofen and etodolac) [13,15,16,19].

Animal studies

Male Sprague–Dawley rats (250–300 g) were

dosed intraperitoneally with either *S*-ibuprofen, *S*-ketoprofen, *S*-etodolac, *R*-etodolac or *S*-flurbiprofen (100 mg/kg) in PEG-400 (total volume 0.3 ml). Four hours following the drug administration the animals were anaesthetized using diethyl ether followed by methoxyflurane (Metofane, Pitman-Moore, Mississauga, Canada) and whole blood samples (5 ml) were collected by cardiac puncture. The specimen was immediately transferred to heparinized tubes, centrifuged at 1800 *g*, and the plasma frozen (−10°C) in clean glass tubes until analysis.

In vitro effect of ethyl chloroformate concentration on the extent of racemization

Individual solutions of the *R* and *S* enantiomers (≈1 mg/ml) of ibuprofen, ketoprofen, etodolac and flurbiprofen were prepared in 50 m*M* TEA in acetonitrile. Aliquots (20 μl) of each solution were made up to a volume of 200 μl with 50 m*M* TEA in acetonitrile. Ethyl chloroformate solution (6 or 60 m*M* in acetonitrile) was then added to each solution, to provide ethyl chloroformate concentrations of 0.43, 0.86,1.71, 4.3, 8.6, 17.1 and 43 m*M* in a constant volume, and vortex-mixed for 30 s. Finally, as outlined in the analytical methods [13,15,16,19] 50 μl of either *R*-(+)-α-phenylethylamine [1 *M* in TEA–acetonitrile (2:8, v/v)] or L-leucinamide [0.5 *M* in TEA–acetonitrile (2:8, v/v)] were added to form diastereomers. The derivatization reaction was terminated by the addition of 200 μl of water. Aliquots of 20 μl were injected into the HPLC system and the ratios of the peak areas corresponding to the diastereomers of the *R* and *S* compounds were calculated.

RESULTS AND DISCUSSION

All of the enantiomers provided were used without further purification. Analysis using the methods outlined previously [13,15,16,19] demonstrated that there was an apparent contamination of each enantiomer with <7% of the antipode. Therefore, in order to minimize the amount of the stereochemical contaminant present, plasma samples were collected at 4 h after dosing. This time delay would allow clearance of a significant portion of stereochemical contaminant present in the body [19,23,24] and provide samples containing no antipode. The *R* enantiomer of all the drugs used, except etodolac, have either greater or equal clearance than their respective *S* enantiomer [19,22,23,24]. The clearance of *R*-etodolac, on the other hand, is much slower than that of the *S* antipode [22]. Thus, even a small proportion of the *R* enantiomer present in the administered dose of *S*-etodolac as contaminant would not be cleared within 4 h. Further, *R*-etodolac does not appear to be bioinverted to the *S* enantiomer. Consequently, a plasma sample collected 4 h after administration of *R*-etodolac is likely to be stereochemically more refined than that taken after a dose of the *S* enantiomer. Therefore *R*-etodolac was also administered to rats .

Representative chromatograms from the *in vivo* samples for each drug are shown in Fig. 1. In each case the concentration of the converted enantiomer was above the minimum quantifiable concentration of the assay employed. The concentrations for each enantiomer and percentage of stereochemical conversion (estimated as the ratio of the enantiomer concentrations) are presented in Table I. In all cases the conversion of the *in vivo* samples was less than the percentage of the stereochemical purity found in the powders as directly determined *in vitro*. Therefore we can estimate the stereochemical purity of the original enantiomers as the difference between the percentage of the antipode found in the pure powders and the conversion observed from the *in vivo* samples. By this method the purity of the original enantiomers can be estimated as: >99% for *S*-ibuprofen, 98.9% for *S*-ketoprofen, 99% for *S*-flurbiprofen and >99% for *R*- and *S*-etodolac. Our observation, which is in agreement with that of Ahn *et al.* [20], demonstrates that chiral conversion during the derivatization procedure does occur.

To examine the cause(s) of conversion we examined the effect of changing the concentration of ethyl chloroformate during the derivatization reaction. The results of these studies for both the

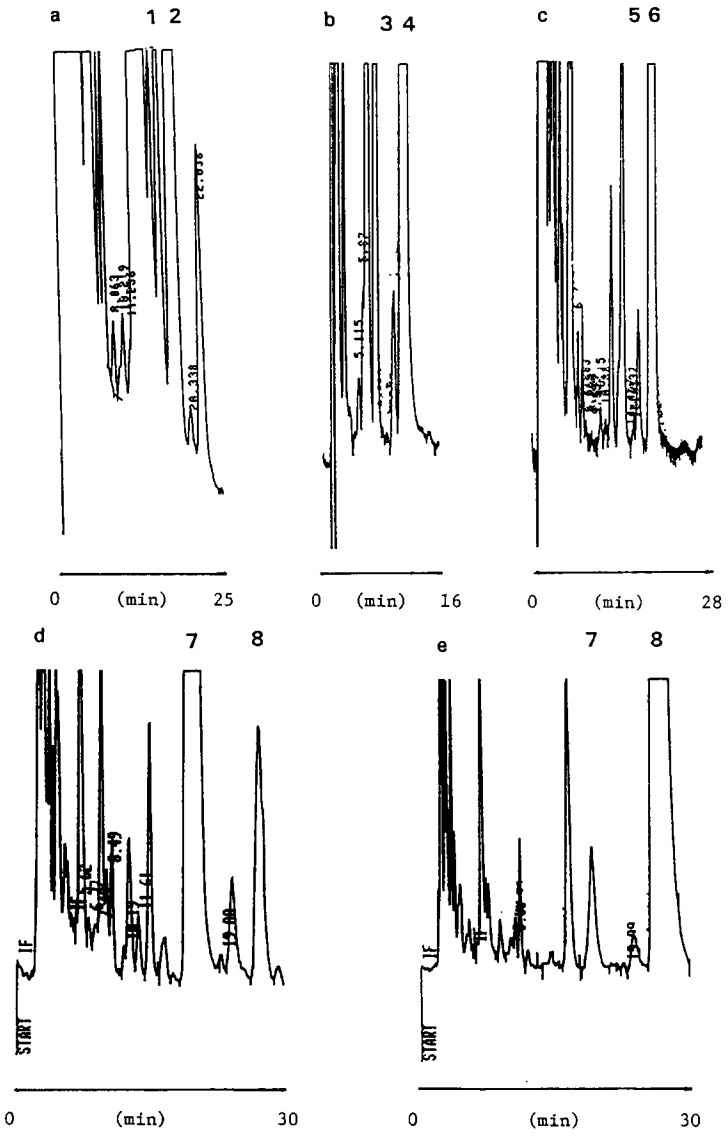

Fig. 1. Representative chromatograms obtained from rats dosed intraperitoneally (100 mg/kg) with (a) *S*-ibuprofen, (b) *S*-ketoprofen, (c) *S*-flurbiprofen, (d) *S*-etodolac and (e) *R*-etodolac. Peaks: 1 = *R*-ibuprofen; 2 = *S*-ibuprofen; 3 = *R*-ketoprofen; 4 = *S*-ketoprofen; 5 = *R*-flurbiprofen; 6 = *S*-flurbiprofen; 7 = *S*-etodolac; 8 = *R*-etodolac. Chromatographic conditions are outlined in the Experimental section.

R and *S* enantiomers are presented in Fig. 2. With the exception of etodolac, for each drug studied there was a marked increase in the extent of stereochemical conversion as the concentration of ethyl chloroformate decreased. In all cases there was no evidence of the presence of underivatized drug. Thus as in the analytical methods used [13,15,16,19] the extent of derivatization in the present studies is also close to 100%. The role of ethyl chloroformate in the conversion of the enantiomers is not certain but the decrease in conversion with increasing ethyl chloroformate concentration may suggest that at low concentrations a side-reaction resulting in conversion oc-

TABLE I

MAXIMAL EXTENT OF STEREOCHEMICAL CONVERSION OF *S* ENANTIOMERS OF VARIOUS NSAIDs BY ETHYL CHLOROFORMATE DETERMINED FROM THE *IN VIVO* STUDIES

Drug	Concentration[a] (μg/ml)		Percent conversion
	R	*S*	
S-Ibuprofen	1.8 ± 1.0	60.5 ± 13.4	2.70 ± 1.10
S-Ketoprofen	0.6 ± 0.4	75.7 ± 26.8	0.83 ± 0.29
S-Flurbiprofen	8.6 ± 6.0	137.8 ± 58.2	5.80 ± 1.60
S-Etodolac	0.4 ± 0.46	39.3 ± 26.4	1.20 ± 0.10
R-Etodolac	606 ± 572	7.7 ± 9.7	1.02 ± 0.44

[a] Concentrations are reported as mean ± standard deviation from duplicate determinations of samples from three rats for each drug.

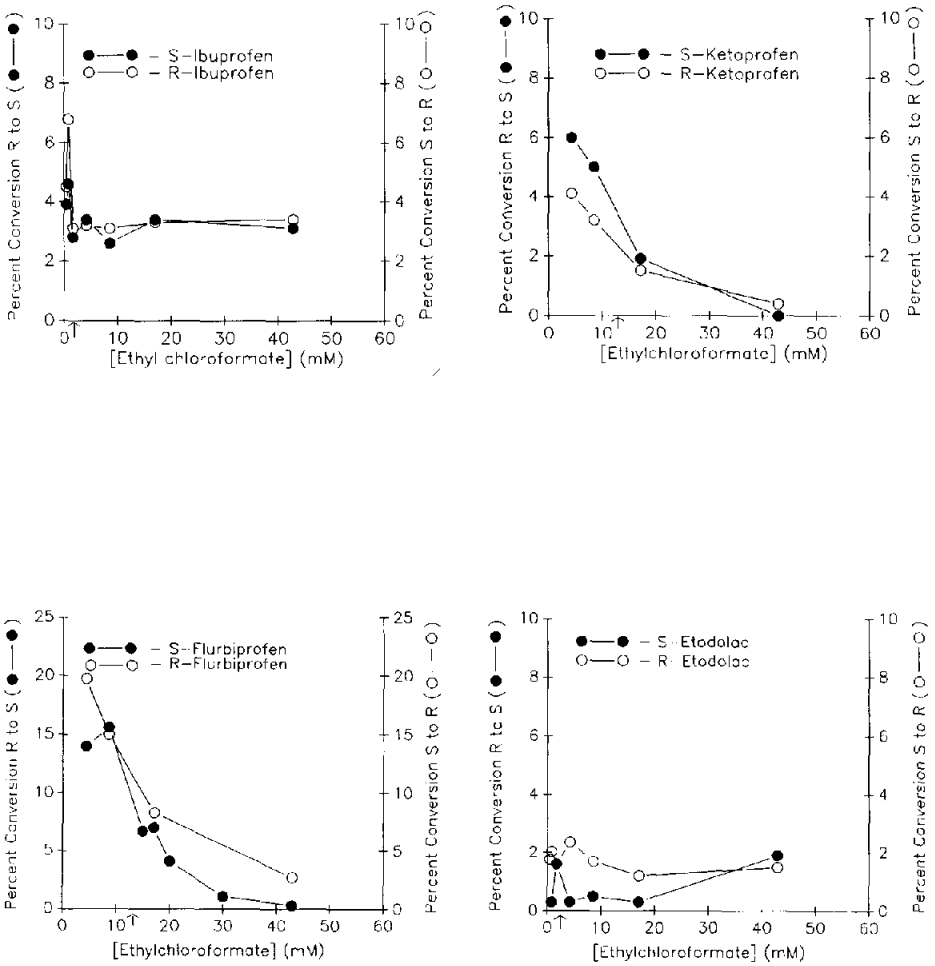

Fig. 2. Dependence of the extent of inversion of the *R* and *S* enantiomers of ibuprofen, ketoprofen, flurbiprofen and etodolac on the concentration of ethyl chloroformate in the derivatization reaction. The arrow indicates the concentration of ethyl chloroformate in the published methods. In each case the solid circles denote the percent of *R* enantiomer present after derivatization of the *S* enantiomer while the open circles denote the percent of *S* enantiomer present after derivatization of the *R* enantiomer. Each point is the mean of duplicate determinations.

curs, but when the concentration of ethyl chloroformate is increased this competing reaction is overwhelmed. It is interesting to note that in the cases of ketoprofen and flurbiprofen for which L-leucinamide was used as the derivatizing reagent there was greater conversion than for etodolac and ibuprofen with *R*-(+)-α-phenylethylamine as reagent. However, the chemical properties of the drug as well as the derivatizing reagent used may also play a role in the extent of conversion observed.

In the published assay methods [13,15,16,19] the concentration of ethyl chloroformate is such that the extent of conversion that would be produced is between 5.8% (*S*-flurbiprofen) and 1.0% (R-etodolac) (Table I). In addition, it can be seen (Fig. 2) that both the *R* and *S* enantiomers behave similarly in that the extent of conversion does not appear to be stereoselective. Therefore, it is reasonable to assume that chiral conversion will have little effect on the analytical accuracy of these methods [13,15,16,19] when the difference in enantiomer concentrations are not substantial. Chiral conversion, however, will have a significant influence on the results of studies where a single enantiomer is administered or the concentrations of the enantiomers differ greatly. It should also be noted that, using the methods examined here, the extent of conversion is relatively small and can be accurately estimated. However, there has been no examination of possible sources of error in methods using either chiral mobile phase additives or chiral stationary phases. Despite claims that these methods are "direct", there has not been extensive examination of the effects of either the stereochemical purity of the reagents/stationary phase used nor the stereochemical stability of the reagents/stationary phase under experimental conditions.

In summary, the present study demonstrates that, as initially suggested by Ahn *et al.* [20], ethyl chloroformate does induce stereochemical conversion of the drugs studied and that the extent of inversion is dependent on the concentration of ethyl chloroformate in the derivatization reaction. However, using the published analytical methods [13,15,16,19] for the drugs studied the absolute error caused ranges between 1.0 and 5.8%. The conversion may be minimized by using concentrations of ethyl chloroformate in the derivatization reaction greater than 17 m*M*. Furthermore, since the extent of stereochemical conversion is equivalent between the enantiomers this factor would introduce only minimal error when the concentrations of the enantiomers are close to one another. Stereochemical conversion may, however, be a significant problem in situations where the enantiomers have much different concentrations. Thus it is necessary that the degree of stereochemical conversion be evaluated as part of the validation process when developing assay methodology employing chiral derivatization.

REFERENCES

1 A. M. Krstulovic, *J. Chromatogr.*, 488 (1989) 53.
2 R. Dennis, *Pharm. Int.*, October (1986) 246.
3 J. Gal, in I. W. Wainer and D. E. Drayer, (Editors), *Drug Stereochemistry: Analytical Methods and Pharmacology*, Marcel Dekker, New York, 1985, p. 78.
4 A. J. Hutt and J. Caldwell, *J. Pharm. Pharmacol.*, 35 (1983) 693.
5 A. J. Hutt and J. Caldwell, *Clin. Pharmacokin.*, 9 (1984) 371.
6 F. Jamali, *Eur. J. Drug Metab. Pharmacokin.*, 13 (1988) 1.
7 F. Jamali, *J. Rheumatol.*, 15 (Suppl. 17) (1988) 71.
8 E. J. D. Lee, K. M. Williams, G. G. Graham, R. O. Day and G. D. Champion, *J. Pharm. Sci.*, 73 (1984) 1542.
9 J.-M. Maitre, G. Boss and B. Testa, *J. Chromatogr.*, 299 (1984) 397.
10 S. Bjorkman, *J. Chromatogr.*, 339 (1985) 339.
11 B. C. Sallustio, A. Abas, P. J. Hayball, Y. J. Purdie and P. J. Meffin, *J. Chromatogr.*, 374 (1986) 329.
12 A. Averginos and A. J. Hutt, *J. Chromatogr.*, 415 (1987) 75.
13 R. T. Foster and F. Jamali, *J. Chromatogr.*, 416 (1987) 388.
14 R. Mehvar and F. Jamali, *Pharm. Res.*, 5 (1988) 53.
15 B. W. Berry and F. Jamali, *Pharm. Res.*, 5 (1988) 123.
16 F. Jamali, R. Mehvar, C. Lemko and O. Eradiri, *J. Pharm. Sci.*, 77 (1988) 963.
17 F. Jamali, F. M. Pasutto and C. Lemko, *J. Liq. Chromatogr.*, 12 (1989) 1835.
18 W. T. C. Liang, D. R. Brocks and F. Jamali, *J. Chromatogr.*, 577 (1992) 317.
19 M. R. Wright, S. Sattari, D. R. Brocks and F. Jamali, *J. Chromatogr.*, 583 (1992) 259.
20 H. Y. Ahn, S. T. Wu, G. K. Shiu, T. D. Doyle, C. A. Brunner and W. F. Trafton, *Pharm. Res.*, 8 (Suppl.) (1992) S31.
21 N. Muller, E. Payan, F. Lapicque, B. Bannwarth and P. Netter, *Fundam. Clin. Pharmacol.*, 4 (1990) 617.

22 D. R. Brocks and F. Jamali, *Drug Metab. Dispos.*, 18 (1990) 471.
23 F. Jamali, B. W. Berry, M. Tehrani and A. S. Russell, *J. Pharm. Sci.*, 77 (1988) 666.
24 R. T. Foster and F. Jamali, *Drug Metab. Dispos.*, 16 (1988) 529.