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Enantioselective determination of dimethindene in urine after oral administration of racemic dimethindene[a]

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ABSTRACT

A sensitive high-performance liquid chromatographic determination of dimethindene and its metabolite N-demethyldimethindene in urine has been developed. The quantitative analysis was followed by determination of the enantiomeric ratio of dimethindene on an α_1-AGP column (EnantioPac®). The urinary data for nine volunteers after oral administration of racemic dimethindene are presented. The isolation, identification and synthesis of the metabolite N-demethyldimethindene are reported.

INTRODUCTION

Dimethindene maleate, N,N-dimethyl-3-[1-(2-pyridinyl)ethyl)]-1*H*-indene-2-ethanamine maleate, is an antihistaminic drug, which is used in therapy as the racemate. Daily doses of only 3–6 mg are required. Separation of the enantiomers can be achieved by fractional crystallization with tartaric acid [1,2]. *In vitro* experiments showed that the enantiomers of dimethindene differ in their pharmacological activity. For *R*-(−)-dimethindene a greater H_1-antihistaminic activity was found [3]. Stereoselective disposition of enantiomers also can result in different pharmacological profiles owing to different rates of absorption or stereoselective metabolism, distribution or clearance [4]. To date no information is available on the disposition kinetics of individual dimethindene isomers in humans or animals after administration of the racemic drug. A gas chromatographic (GC) method for determination of racemic dimethindene with a limit of detection of 10 ng/ml urine has been reported [5,6]. Recently, an enzyme-linked immunosorbent assay with a limit of detection of 0.1 ng/ml dimethindene has been developed [7]. Because of interferences this assay is only applicable for serum.

This paper describes a method for the determination of dimethindene and its metabolite N-demethyldimethindene by high-performance liquid chromatogra-

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phy (HPLC) on a reversed-phase column and the analysis of the enantiomeric ratio of dimethindene by HPLC on a chiral stationary phase. The urinary data of nine volunteers after oral administration of racemic dimethindene are presented. The isolation, identification and synthesis of N-demethyldimethindene are reported.

EXPERIMENTAL

Chemicals

Dimethindene maleate was obtained from Zyma (Nyon, Switzerland). Fendiline hydrochloride was obtained from Thiemann Arzneimittel (Waltrop, Germany). Acetonitrile, 2-propanol and *n*-hexane were LiChrosolv® reagents from Merck (Darmstadt, Germany). Vinyl chloroformate was purchased from Merck and used without further purification. 1,8-Bis(dimethylamino)naphthalene was obtained from Fluka, (Buchs, Switzerland). NADPH and dichlorodimethylsilane were purchased from Sigma (Deisenhofen, Germany). The other chemicals used were of analytical grade.

Apparatus

A Varian 5000 liquid chromatograph with a variable-wavelength detector 655A-22 (Merck-Hitachi) and a D-2000 chromato-integrator (Merck-Hitachi) were used.

The semipreparative column was a LiChrospher® 60 CN (10 μm particle size, 250 mm × 8 mm I.D., Merck), with a 30 mm × 4 mm I.D. guard column. The mobile phase was 0.02 *M* KH_2PO_4 acetonitrile–water (60:32:8, v/v). The flow-rate was 2.5 ml/min, the column temperature 30°C, and the detection wavelength 254 nm.

The analytical column was a LiChrospher 60 CN (10 μm particle size, 250 mm × 4 mm I.D., Merck) with a 30 mm × 4 mm I.D. guard column. The mobile phase and the detection wavelength were the same as for the semipreparative column. The column temperature was 25°C and the flow-rate 1.0 ml/min.

The chiral column was an α_1-acid glycoprotein cartridge (100 mm × 4 mm I.D., EnantioPac®, LKB) with a LiChrospher® NH_2 guard column (10 μm particle size, 30 mm × 4 mm I.D.). The mobile phase was 0.02 *M* phosphate buffer (pH 7.0) containing 5.0% (v/v) 2-propanol. The flow-rate was 0.3 ml/min, the column temperature 22°C, and the detection wavelength 254 nm.

Mass spectra were obtained with a Finnigan Model MAT 312, operated at 70 eV in the chemical ionization mode with ammonia as reagent gas. pH measurements were carried out with a pH-Digi, WTG (Weilheim, Germany).

Isolation of N-demethyldimethindene

Dimethindene (28 μmol) was incubated with rat liver microsomes (17 mg protein) obtained from male Sprague–Dawley rats that had been pretreated with

phenobarbitone (50 mg/kg). The microsomes were prepared according to the method of Dayer *et al.* [8]. Each incubation mixture contained 1–2 μmol of dimethindene, 0.9 mg of protein, 2 μmol of NADPH and 12 μmol of $MgCl_2$ in 6 ml of Tris buffer (pH 8). Incubation was performed at 37°C for 60 min while gently stirring the mixture. After extraction with *n*-pentane at pH 11, the metabolite was further purified by semipreparative HPLC. The conditions used are given under *Apparatus*. The fractions of interest were extracted with *n*-hexane, and a mass spectrum of the isolated compound was recorded.

Synthesis of N-demethyldimethindene

The N-demethylation of dimethindene was carried out by excluding light and moisture. Dimethindene base (3.1 mmol) and 1,8-bis-(dimethylamino)naphthalene (0.31 mmol) were dissolved in 1,2-dichloroethane (15 ml). The reaction mixture was cooled to −5 to 10°C, and vinyl chloroformate (3.1 mmol) was added with stirring. After 15 min the mixture was refluxed at 60–70°C for 45 min, cooled to room temperature and concentrated. The carbamate was purified by flash chromatography over a silica gel column with *n*-hexane–ethyl acetate (50:50, v/v) as eluent. The eluent was removed under reduced pressure, and an oily residue was obtained (2.8 mmol, 90.3%). The carbamate (3.2 mmol) was dissolved in methylene chloride (15 ml), and a slow stream of hydrogen chloride was bubbled through the solution for 30 min at room temperature. Solvent evaporation was followed by addition of methanol and heating at 70°C for 1 h. The hydrochloride was converted into the maleate salt and recrystallized from diethyl ether–ethanol. The yield was 56.4% (1.83 mmol). N-Demethyldimethindene maleate was characterized by mass spectrometry (MS), NMR, IR spectroscopy and elemental analysis.

Extraction procedure

A 0.5-ml volume of 25% aqueous ammonia was added to each urine sample (4.0 ml). Dimethindene and N-demethyldimethindene were then extracted twice with 4.0 ml of *n*-hexane for 15 min. The tubes were centrifuged for 20 min, and two 3.0-ml volumes of the organic phase were transferred to a flask and evaporated to dryness. The residue was dissolved in 1.0 ml of *n*-hexane and evaporated to dryness under nitrogen. The residue was dissolved in 50 μl of standard solution (54.8 μg of fendiline per ml of 0.02 *M* KH_2PO_4), of which 40 μl were injected into the RP column. Glassware used for concentration was cleaned with chromic sulphuric acid, washed with distilled water and dried; the surface of the glass was deactivated with dichlorodimethylsilane.

Calibration curve, assay precision and recovery from urine

To 4.0 ml of blank urine, 12, 24, 48, 96, 192, 484, 968 and 2416 ng of dimethindene were added and extracted as described above. Each concentration was prepared six to eight times. The calibration curve was obtained by plotting the ratio

of the peak area of dimethindene to that of the standard against the known concentrations of dimethindene. The recovery of dimethindene was also calculated from these data. The calibration curve and recovery of N-demethyldimethindene were obtained in the same way: 25, 60, 125 and 313 ng of N-demethyldimethindene were added to 4.0 ml of blank urine, and processed as described.

Determination of the enantiomeric ratio in urine

Urine samples were extracted as described above and separated by HPLC on the RP-CN column. Dimethindene fractions were collected, and acetonitrile was evaporated under a stream of nitrogen. Fractions were extracted with *n*-hexane as described for urine samples. The residue was dissolved in 50 μl of 0.02 *M* KH_2PO_4 solution, and 45 μl were injected onto the chiral column.

For calibration, pure enantiomers were combined to obtain mixtures of known optical purity, which were then analysed.

In vivo study

Eight healthy volunteers received 4 mg of dimethindene as an aqueous solution (Fenistil® Tropfen). Urine was collected during the following intervals: 0–4, 4–14, 14–18, 18–22, 22–26 and 26–29 h. The volume and pH were measured, and 30 ml were stored at −20°C until analysis. A 3-mg sample of dimethindene (coated pill) was administered to a healthy volunteer who had taken five oral doses of 400 mg of ammoniumchloride the day before and three additional doses on the day of administration in order to adjust the urinary pH to acidic. Urine samples were collected at 1-h intervals up to 8 h after administration and then during the following intervals: 8–10, 10–12, 12–16.5, 16.5–18, 18–23.5, 23.5–27 h.

RESULTS AND DISCUSSION

Isolation and synthesis of N-demethyldimethindene

After extraction of human urine samples with *n*-hexane at pH 11 following administration of dimethindene, an unknown peak was detected (Fig. 1). This peak was also observed after incubation of dimethindene with rat liver microsomes. Sufficient amounts of this metabolite for a mass spectral analysis were obtained by incubation of dimethindene with rat liver microsomes, followed by extraction with *n*-hexane and isolation by HPLC. The mass spectrum with chemical ionization showed the molecular ion peak at *m/z* 279, which indicated an N-demethylated structure. The structure was confirmed by synthesis of N-demethyldimethindene.

For the selective N-demethylation of tertiary amines vinyl chloroformate is a suitable reagent, which also allows the subsequent cleavage of the carbamate intermediate under mild conditions [9,10]. In the presence of 1,8-bis(dimethylamino)naphthalene as proton scavenger, dimethindene as the free base reacts very selectively with vinyl chloroformate to give the corresponding N-demethyl-

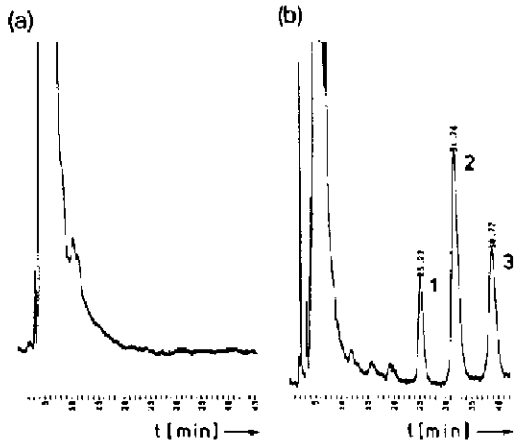

Fig. 1. Chromatograms of (a) blank urine and (b) a urine sample after oral administration of 4 mg of dimethindene. Peaks: 1 = metabolite, N-demethyldimethindene; 2 = dimethindene; 3 = internal standard, fendiline. Chromatogrpahic conditions: column, LiChrospher 60 CN (250 mm × 4 mm I.D.) with guard column; mobile phase, 0.02 *M* KH_2PO_4–acetonitrile–water (60:32:8, v/v); flow-rate 1 ml/min.

ated vinyl carbamate. The vinyl carbamate is cleaved by addition of hydrogen chloride in methylene chloride, followed by warming of the reaction mixture in methanol (Fig. 2). The existence of an N-demethylated metabolite of dimethindene was first suggested by Maurer and Pfleger [11], based on gas chromatographic–mass spectrometric (GC–MS) analysis of a human urine sample after acetylation.

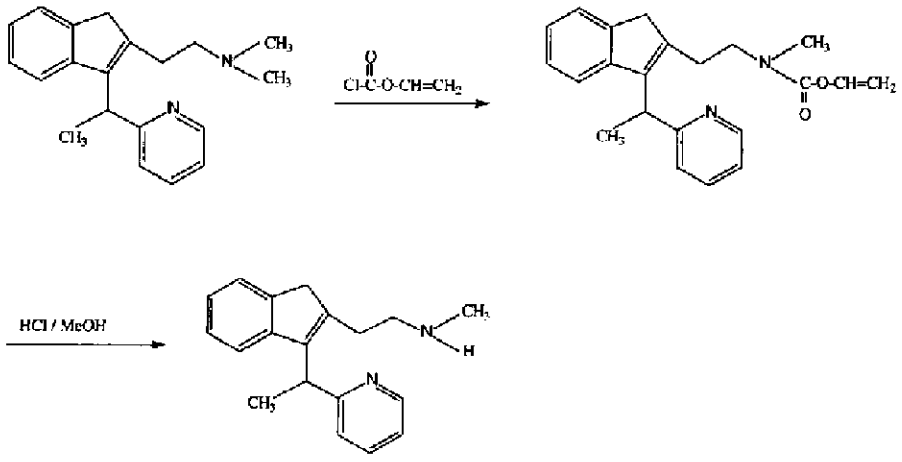

Fig. 2. Synthesis of N-demethyldimethindene.

Quantitative determination

For the determination of dimethindene and N-demethyldimethindene by HPLC we used a cyano column in the reversed-phase mode. This gave a better separation of dimethindene and N-demethyldimethindene than C_8 or C_{18} phases, and with reasonable retention times. In addition, no interferences occurred with this column after the extraction of urine samples.

Assay precision and recovery of dimethindene and N-demethyldimethindene. Table I shows the results obtained with urine samples spiked with dimethindene concentrations from 3 to 604 ng/ml and N-demethyldimethindene concentrations from 6 to 78 ng/ml. Each concentration was prepared six to eight times and analysed on different days. Because no dimethindene analogues were available, we had to find another suitable internal standard (I.S.). Among antihistamines with lipophilic and basic properties comparable with dimethindene, we chose fendiline [N-(1-phenylethyl)-3,3-diphenylpropylamine] as the I.S. This compound has a retention time greater than that of dimethindene, and therefore it did not interfere with the more hydrophilic metabolites of dimethindene on the reversed-phase column. Reproducible results were achieved only when adding the I.S. after the extraction. The extraction of urine with *n*-hexane gives clean extracts with no interferences present (Fig. 1).

Deactivation of the surface of the glassware used was necessary to obtain reproducible results at very low concentrations of dimethindene and N-demethyldimethindene.

The calibration curve for the analysis of dimethindene in urine at 0–600 ng/ml, as a function of peak-area ratio, gives the equation (peak area of dimethindene/peak area of I.S.) = (0.034 × concentration of dimethindene) + 0.005. The correlation coefficient of 0.9999 indicates good linearity of the data. The cali-

TABLE I

ASSAY PRECISION AND RECOVERY

Dimethindene				N-Demethyldimethindene			
Amount added (ng/ml)	Amount found (ng/ml)	S.D. (ng/ml)	Recovery (%)	Amount added (ng/ml)	Amount found (ng/ml)	S.D. (ng/ml)	Recovery (%)
3	3.1	0.3	102.6	6	3.1	0.5	49.4
6	6.2	0.5	102.6	12	6.7	3.0	53.2
12	12.3	1.0	102.1	31	16.9	1.3	53.9
24	23.8	1.4	98.5	78	47.5	0.5	60.6
48	48.9	4.2	101.1				
121	121.3	4.6	100.4				
242	246.2	20.8	101.9				
604	609.6	42.1	100.9				

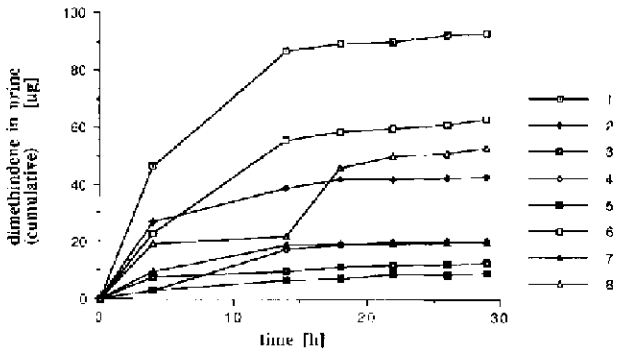

Fig. 3. Cumulative excretion curves of dimethindene in urine after oral administration of 4 mg of dimethindene to eight healthy volunteers.

bration curve for the analysis of the metabolite is (peak area of N-demethyldimethindene/peak area of I.S.) = (0.024 × concentration of N-demethyldimethindene) − 0.05, with a correlation coefficient of 0.999. In the case of N-demethyldimethindene, the recovery decreases at lower concentrations, hence the accuracy at the lowest concentration of N-demethyldimethindene was 9.2%.

Dimethindene in human urine. Fig. 3 shows the cumulative excretion curves of dimethindene in urine after oral administration of 4 mg of dimethindene to eight volunteers. After 29 h, an average of 0.98% of the administered dose of unmetabolized dimethindene was excreted. The variations between the volunteers were quite large (0.2% as the lowest and 2.3% as highest value). One reason for this finding is that the urinary pH of the volunteers was not standardized by means of oral administration of ammonium chloride. As is well known for basic drugs, the urinary excretion depends on the pH of the urine [12]. Within the first interval (0–4 h) a linear dependency of dimethindene excreted (log µg) *versus* the pH of urine was observed (Fig. 4).

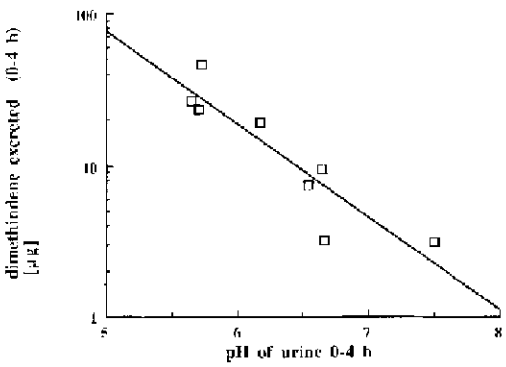

Fig. 4. pH dependency of the urinary excretion of dimethindene after oral administration of dimethindene.

TABLE II

DIMETHINDENE AND N-DEMETHYLDIMETHINDENE IN HUMAN URINE AFTER ORAL ADMINISTRATION OF DIMETHINDENE

Amount of dose, 3 mg.

Time (h)	Urine pH	Dimethindene			N-Demethyldimethindene	
		Concentration (ng/ml)	Excreted per hour (μg)	Cumulative excretion (μg)	Concentration (ng/ml)	Cumulative excretion (μg)
0–1	5.0	7	0.9	0.9		
1–2	5.2	58	20.1	21.0	16	5.6
2–3	4.9	172	19.3	40.3	71	13.5
3–4	5.0	96	13.3	53.5	83	25.0
4–5	4.8	120	9.9	63.4	131	35.8
5–6	4.9	217	8.5	71.9	340	49.1
6–7	5.3	85	3.6	75.5	267	60.3
7–8	[a]	50	2.3	77.8	194	69.1
8–10	5.5	15	1.8	81.5	59	83.6
10–12	5.5	23	2.2	85.9	73	97.3
12–16.5	7.1	3[b]	0.3	87.3	9	100.8
16.5–18	—[a]	2[b]	0.3	87.7	11	103.4
18–23.5	6.2	3	0.1	88.3	27	107.9
23.5–27	5.0	24	0.6	90.5	61	113.6

[a] Not determined.

[b] 16 ml instead of 4 ml were used for extraction.

The cumulative excretion curves of N-demethyldimethindene look quite similar to those of dimethindene, and 0.3–3.2% of the administered dose of dimethindene was excreted after 29 h (average 1.43%).

Table II shows the urinary data for one volunteer after administration of 3 mg of dimethindene and ammonium chloride to lower the urinary pH. The total amount of free dimethindene excreted in the urine was 3% of the administered dose, about three times higher than the average of the eight volunteers (see above). The data in Table II also show the dependence of the urinary excretion on the pH of the urine. At 12 h after administration, the pH had increased to 7.1 and the amount excreted per hour had dropped from 2.2 to 0.3 μg.

Determination of the enantiomeric ratio of dimethindene

Sample preparation and calibration. For the extraction of urine samples the non-polar solvent *n*-hexane was chosen, in order to obtain clean samples and to prevent the extraction of most dimethindene metabolites. N-Demethyldimethindene, however, was present in *n*-hexane extracts. This metabolite interferes with the resolution of dimethindene on the chiral column. N-Demethyldimethindene is

also separated on the α-AGP column but the (−)-enantiomers of both compounds are coeluted. Therefore it was necessary to separate dimethindene from its metabolite. It was not possible to couple the chiral column and the reversed-phase guard column that separates dimethindene and N-demethyldimethindene, because the protein column only allows aqueous buffers as mobile phase with small amounts of organic modifier. Therefore, we decided to isolate dimethindene by HPLC on a cyano column and then separate its enantiomers on the α-AGP column. This method allows the selective isolation of dimethindene with almost quantitative recovery. The dimethindene fractions were then extracted with *n*-hexane, as described for the urine samples. More convenient would have been lyophilization of dimethindene fractions, but this was not possible because the remaining buffer substances were insoluble in 50 μl of water.

For calibration of the assay, the pure enantiomers were combined in order to obtain mixtures with known enantiomeric ratios. Pure enantiomers of dimethindene were obtained by fractional crystallization of their diastereomeric tartrate salts. Different concentrations of each mixture were chromatographed on the α-AGP column, and calibration curves for each enantiomer were obtained.

Enantiomeric ratio of dimethindene in human urine. Fig. 5 shows the separation of dimethindene isolated from the urine of a volunteer after the oral adminis-

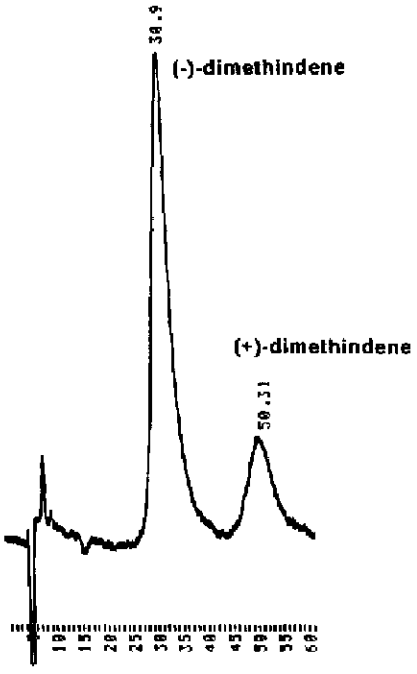

Fig. 5. Chromatogram of dimethindene isolated from human urine. Chromatographic conditions: EnantioPac cartridge (100 mm × 4 mm I.D.) with LiChrospher NH_2 guard-column; mobile phase, 0.02 *M* phosphate buffer (pH 7.0) with 5% 2-propanol (v/v); flow-rate, 0.3 ml/min.

TABLE III

DIMETHINDENE ENANTIOMERS IN HUMAN URINE

Time (h)	Enantiomeric ratio (%)		Cumulative excretion (μg)	
	R-(−)	*S*-(+)	*R*-(−)	*S*-(+)
0–1	31.2	68.8	0.3	0.6
1–2	37.6	62.4	7.8	13.1
2–3	49.6	50.4	17.4	22.8
3–4	56.8	43.2	24.9	28.6
4–5	56.6	43.4	30.5	32.9
5–6	57.7	42.3	35.4	36.5
6–7	63.7	36.3	37.7	37.8
7–8	65.8	34.2	39.2	38.6
8–10	62.0	38.0	41.5	40.0
10–12	67.7	32.3	44.5	41.4
12–16.5	54.7	45.3	45.3	42.1
16.5–18	63.5	36.5	45.5	42.2
18–23.5	58.7	41.3	45.8	42.4
23.5–27	54.2	45.8	47.0	43.4

tration of 3 mg of racemic dimethindene and ammonium chloride. Table III shows the enantiomeric ratios of dimethindene in the urine of this volunteer. The urinary excretion of the enantiomers was then calculated using the data obtained with the non-stereoselective method (Tables II and III). In the first 2 h after administration, more *S*-(+)-dimethindene was excreted. In the third interval,

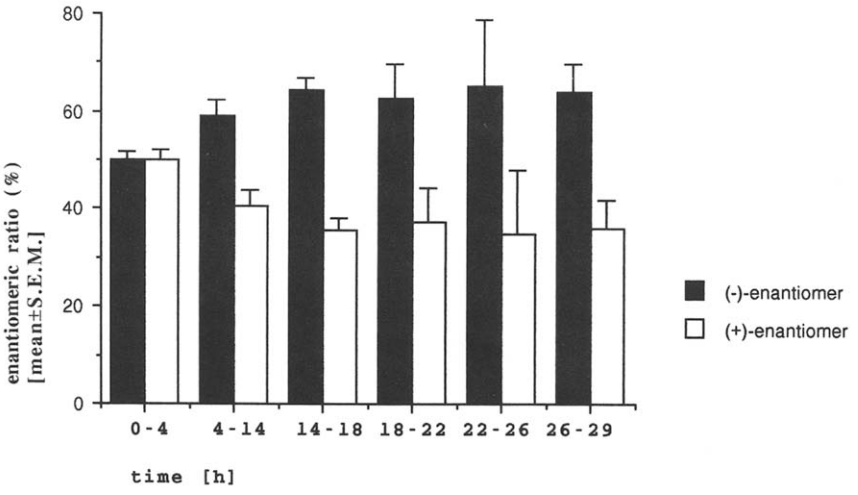

Fig. 6. Enantiomeric ratios of dimethindene in urine after oral administration of 4 mg of racemic dimethindene to eight volunteers. Numbers: 0–4 h, $n = 8$; 4–14 h, $n = 7$ ($p < 0.05$); 14–18 h, $n = 7$ ($p < 0.01$); 18–22 h, $n = 4$; 22–26 h and 26–29 h, $n = 3$

dimethindene was excreted as the racemate. From then on, the excretion of the *R*-(+)-enantiomer was preferred. Overall, slightly more *R*-(−)-dimethindene was excreted. A higher H_1-receptor affinity for the more active (+)-enantiomer could lead to a faster excretion of (+)-dimethindene within the first few hours after administration. The reversal of the enantiomeric ratio may then be due to a stereoselective metabolic reaction of (+)-dimethindene.

Fig. 6 shows the enantiomeric ratios of dimethindene in urine after oral administration of 4 mg of racemic dimethindene to eight volunteers. The urinary excretion pattern of the dimethindene enantiomers is the same as that found after administration of 3 mg of dimethindene to one person (see above): 4 h after administration the enantiomeric ratio found was *ca.* 1.0, but later more (−)-dimethindene was excreted. The differences between the enantiomers are significant within the intervals 4–14 h ($p < 0.05$) and 14–18 h ($p < 0.01$).

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