PHARMACOKINETICS OF LEVOFLOXACIN IN COMPARISON TO THE RACEMIC MIXTURE OF OFLOXACIN IN MAN

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SUMMARY

After oral administration of a single dose of 200 mg of levofloxacin and 400 mg racemic mixture of ofloxacin to 6 healthy male volunteers in a double-blind, randomised cross-over study, concentrations of the unchanged isomers were determined at various times in serum and urine, over 28 hours and 48 hours, respectively. Each dosing was followed by a wash-out period of one week. Ofloxacin concentrations were determined using an enantioselective and a non-enantioselective high pressure liquid chromatography (HPLC) assay. The two measurements obtained were compared by linear distribution independent regression, and were found to be equivalent.

Maximum serum concentration (C_{max}) of levofloxacin after the administration of 200 mg of the levo-isomer was 2.42 mg/l (chiral derivatization HPLC, mean values); the corresponding area under the serum concentration-time curve (AUC_{0-28}) was 17.0 mg x h/l. The corresponding C_{max} values after the administration of 400 mg (±)-isomer (chiral derivatization HPLC and reversed phased HPLC, mean values) were 2.05 mg/l, 1.98 mg/l and 4.41 mg/l for (-)-, (+)- and (±) isomer, respectively. The $AUCs_{0-28}$ were 17.0, 14.6 and 32.7 mg x h/l, respectively. The pharmacokinetics of the (-)- and (+)-isomer were shown to be almost equal. In serum and urine no reracemisation of the

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(-)-isomer to a racemic mixture was observed. General tolerability was good; no side effects were reported.

KEY WORDS

levofloxacin, ofloxacin, racemic mixture, pharmacokinetics

INTRODUCTION

Drug enantiomers can have different pharmacokinetic /1,2/ and pharmacodynamic /3,4/ properties.

Ofloxacin, a gyrase inhibitor with an extremely broad antibacterial spectrum /5-7/, has a chiral C-atom at the C3-position and therefore consists of two optically active isomers /8/. The *in vitro* antibacterial activities of the (-)-form are 8 to 128 times that of (+)-ofloxacin and about twice that of the racemic mixture of ofloxacin against gram-positive and gram-negative bacteria tested /8,9/. The (-)-form is highly active against isolates of *E. coli, Citrobacter freundii, K. pneumoniae, Enterobacter cloacae, H. influenzae* and *B. catarrhalis* with MIC_{90}s ranging from 0.05 to 0.39 mg/l. The (+)-isomer is poorly active against *Staphylococcus* spp., *Streptococcus* spp. and *Enterococcus* spp. with MIC_{90}s ranging from 50 - >100 mg/l. It is active against gram-negative bacterial isolates but the activity is at least 30 times less than that of the (-)-isomer /10/.

The aim of the present study was to investigate the pharmacokinetics of the pure (-)-form[1] in comparison to the racemic mixture, in order to detect possible differences in the pharmacokinetic characteristics. A second goal was to investigate whether an *in vivo* reracemisation from the (-)-form to the racemic mixture takes place in the body.

SUBJECTS AND METHODS

Six healthy male volunteers entered the study. Their mean age was 41 ± 8 years. They had normal body build with a mean weight of 77 ± 7 kg and a mean height of 174 ± 5 cm. All volunteers gave their

[1] 0.3% of the (+)-form was detected as contamination in this form (Lehr, personal communication).

consent in writing after being informed by the physician of the nature, purpose and possible risks of the trial. All volunteers underwent an initial physical examination including standard safety checks that was repeated 24 hours after the last application; there were no pathological findings.

The volunteers received a single oral dose of 200 mg levofloxacin or 400 mg of the racemic mixture in a double-blind, randomised cross-over study design. Each dosing was followed by a wash-out period of one week before the next dosing.

Blood samples were taken from an antecubital vein before dosing and at the following times after dosing: 5 min, 15 min, 30 min, 45 min, 1 h, 1.25 h, 1.5 h, 1.75 h, 2 h, 2.5 h, 3 h, 3.5 h, 4 h, 5 h, 6 h, 7 h, 9 h, 12 h, 14 h, 24 h, 25 h and 28 hours. 7 ml blood (2 aliquots of 1.5 ml of serum) was taken at each sampling time. Urine was collected in 4-hourly fractions for 12 hours, thereafter in 12-hourly fractions for a further 36 hours.

Two HPLC-methods were applied to this study. (-)-Ofloxacin (levofloxacin) and (+)-ofloxacin concentrations in serum and urine were determined using high pressure liquid chromatography (HPLC) after chiral derivatization /11/. In addition, (±)-ofloxacin concentrations were determined using a non-enantioselective HPLC-method /12/.

The imprecision between day of the enantioselective assay determined in the concentration range from 15 ng to 5000 ng per ml serum was between 5.3 and 12.6% for (-)-ofloxacin and between 4.9 and 12.9% for (+)-ofloxacin. In urine, the corresponding values were between 2.9 and 18.8% for (-)-ofloxacin and between 2.9 and 16.1% for (+)-ofloxacin determined in the concentration range from 0.15 to 50 mg/l. The detection limits for (-)- and (+)-ofloxacin in serum were 10 ng/ml and in urine 50 ng/ml and 70 ng/ml, respectively /11/.

The imprecision between day of the non-enantioselective assay determined in the concentration range from 10 ng to 2000 ng per ml serum was between 1.9 and 10.2% for (±)-ofloxacin. In urine, the corresponding values were between 2.1 and 11.1% for (±)-ofloxacin determined in the concentration range from 0.5 to 500 mg/l. The detection limit for (±)-ofloxacin was 4 ng/ml in serum and 200 ng/ml in urine.

The concentration-time profiles of ofloxacin were best described by a two-compartment open model /13/, which was fitted to the data by means of a computer programme for non-linear approximations /14/.

RESULTS

Method comparisons

Measurement comparisons using linear distribution independent regression analysis were calculated for serum and urine according to Passing and Bablok /15/, shown in Figure 1 for serum.

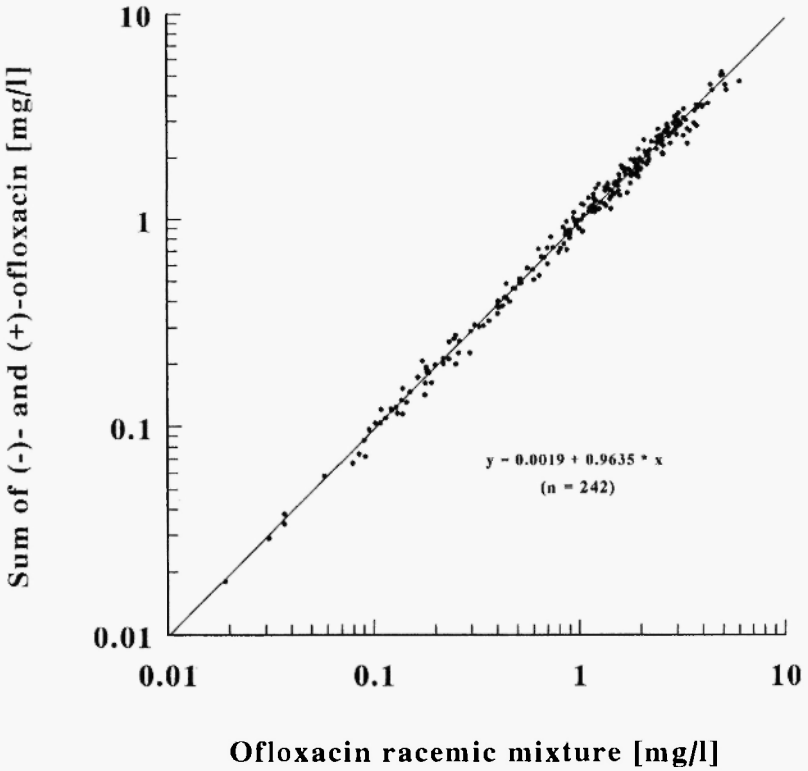

Fig. 1: Measurement comparison for serum between the original HPLC method for the racemic mixture and the sum of the two enantiomers determined by means of the enantiospecific HPLC method.

The sums of (-)- and (+)-forms in both serum and urine were compared directly with the data from original HPLC concentrations. An excellent correspondence ($r = 0.986$ for serum, $r = 0.994$ for urine; $p < 0.001$ for both) between HPLC methods was found; both regression lines are not statistically different from the unity line (slope 1, intercept 0).

Pharmacokinetics

The time courses of ofloxacin concentrations in serum after both dosings are shown in Figures 2 and 3. The corresponding pharmacokinetic data are given in Table 1.

The terminal half-life, AUC values and the urinary recovery of the (+)-isomer were slightly less than the corresponding values of the (-)-form in the 400 mg racemate tablet. The pharmacokinetic variables of 200 mg levofloxacin and 400 mg tablet estimated as (-)-isomer showed no differences at all.

Neither in urine nor serum was there any evidence of re-racemisation of the (-)-isomer to the corresponding (+)-form.

Tolerability

Adverse events did not occur in any subject during the trial period; no clinically relevant changes were noted in haematological, biochemical or urine analyses.

DISCUSSION

Levofloxacin, the S-(-)-isomer of ofloxacin, has an antibacterial activity twice as high /8,9/ and an anti-DNA activity twice as high than ofloxacin, the racemic mixture /16/. In the treatment of complicated urinary tract infections levofloxacin in a dose of 100 mg t.i.d. proved as effective as ofloxacin 200 mg t.i.d. /17/.

The enatioselective disposition of ofloxacin after administration of the racemic mixture has been investigated in animals and man. Okazaki *et al.* /18/ investigated the serum concentration profiles in rat, monkey and dog. They found that in rat the (R)-ofloxacin predominates, whereas the opposite is the case in monkeys. In dogs no differences in the pharmacokinetics of the enatiomers were observed.

The enatioselective disposition of ofloxacin was investigated in healthy subjects after oral administration of 200 mg (±)-ofloxacin /19/.

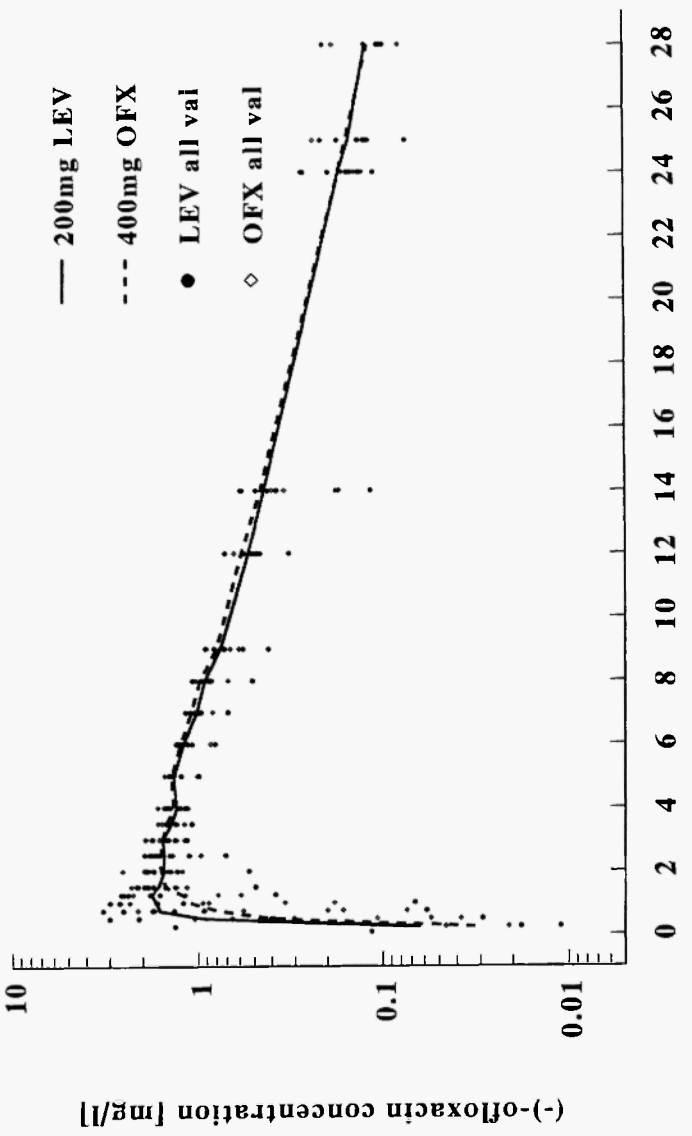

Fig. 2: Mean serum concentration curves of the two ofloxacin formulations administered (200 mg levofloxacin, 400 mg racemic mixture): a) analysed as (-)-isomer by chiral derivatization HPLC; b) analysed as (+)-isomer by chiral derivatization HPLC; c) analysed by reversed phase HPLC.

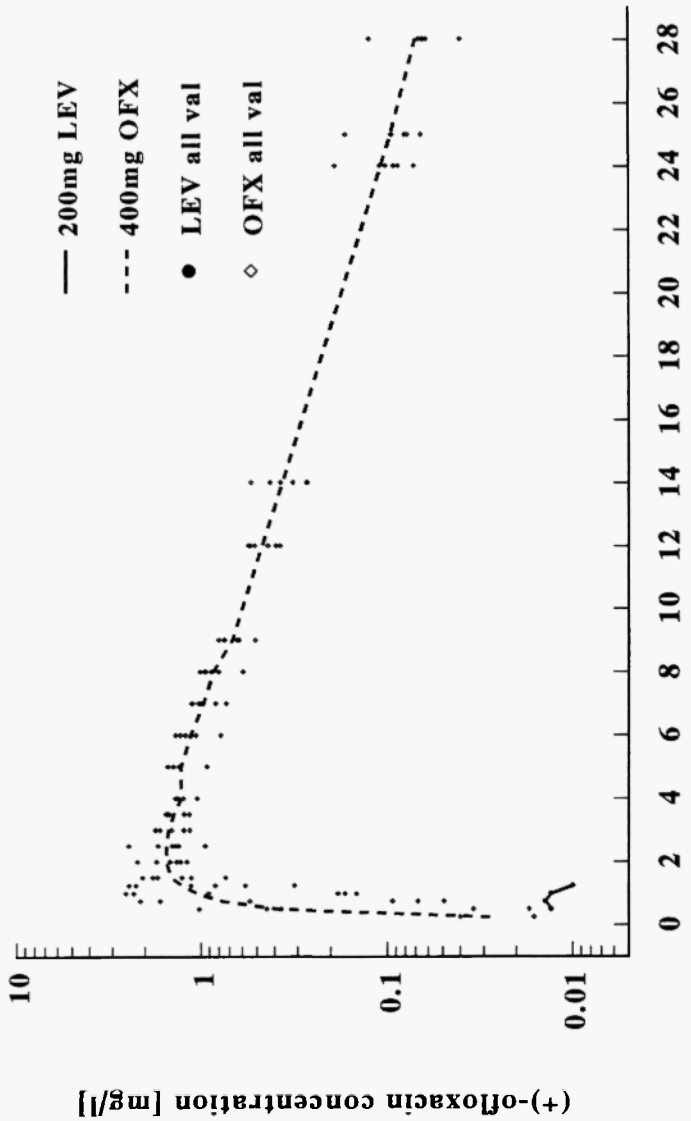

Fig. 3: Mean serum concentration curves of the (-)- and (+)-isomer analysed from the 400 mg racemic mixture.

TABLE 1

Pharmacokinetic data of (-)- and (+)-ofloxacin and the racemic mixture after oral administration of 200 mg levofloxacin or 400 mg of the racemic mixture (mean values ± S.D.)

Variables	200 mg levofloxacin			400 mg racemic mixture		
	chiral derivatization HPLC		reversed phase HPLC	chiral derivatization HPLC		reversed phase HPLC
	(-)-form	(+)-form[2]		(-)-form	(+)-form	
C_{max} [mg/l]	2.42 ± 0.54	0.01	2.50 ± 0.42	2.05 ± 0.56	1.98 ± 0.53	4.41 ± 1.09
C_{12} [mg/l]	0.54 ± 0.10		0.54 ± 0.07	0.59 ± 0.10	0.47 ± 0.08	1.06 ± 0.17
C_{24} [mg/l]	0.17 ± 0.05		0.17 ± 0.04	0.17 ± 0.06	0.11 ± 0.04	0.30 ± 0.10
t_{max} [h]	1.4 ± 0.7	0.8	1.4 ± 0.7	1.8 ± 0.7	1.8 ± 0.7	1.8 ± 0.7
$t_{½\,\beta}$ [h]	6.5 ± 0.9		6.8 ± 0.6	6.5 ± 1.4	7.6 ± 1.2	5.9 ± 0.9
AUC_{0-28} [mg*h/l]	17.0 ± 3.2	0.01	17.1 ± 2.1	17.0 ± 2.6	14.6 ± 2.3	32.7 ± 4.5
				31.6 ± 3.5		
AUC [mg*h/l]	17.9 ± 3.6		18.1 ± 2.4	17.8 ± 3.0	14.8 ± 2.6	34.1 ± 4.7
				32.7 ± 4.0		
CL_{tot}/f [ml/min]	192.3 ± 36.3		186.7 ± 23.7	191.4 ± 32.8	230.4 ± 39.0	198.5 ± 25.1
URINARY RECOVERY						
mg	151.3 ± 16.8	2.9 ± 6.0	182.1 ± 21.5	153.4 ± 18.9	144.4 ± 18.0	337.6 ± 37.9
% of dose	75.7		91.4	38.4	36.1	84.4
				74.5		

[2] See footnote 1

No differences in the peak concentration or time to reach peak concentration were observed, suggesting that the absorption of ofloxacin is not stereoselective in humans; however renal clearance was different. Renal clearance of the R-(+)-isomer was significantly greater than that of the S-(-)-isomer. The half-life of the S-(-)-isomer was statistically significantly longer than that of the R-(+)-isomer.

The results of this study indicate that in the racemic mixture the (+)-isomer possesses a slight trend to a decreased half-life, a slight reduction of the AUC-value and a slight decrease in urinary recovery of the unchanged drug. These differences, however, are minimal and without any relevance with respect to clinical application. The half-lives and urinary recoveries of both formulations are in the same range as reported previously /20-23/.

No differences were seen in the levofloxacin pharmacokinetic variables after the administration of 200 mg of the pure (-)-isomer or 400 mg of the racemic mixture (See Table 1) and no reracemisation occurred in the (-)-isomer to the racemic mixture as shown in the urinary excretion of the drug.

The good antibacterial activity of levofloxacin in comparison to the racemic mixture makes this substance a very interesting new development. However its clinical superiority as compared to the racemate has still to be confirmed in the clinical setting.

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