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Inhibitory Potency of Quinolone Antibacterial Agents against Cytochrome P450IA2 Activity In Vivo and In Vitro

U. FUHR,[1]* E.-M. ANDERS,[1] G. MAHR,[2] F. SÖRGEL,[2] AND A. H. STAIB[1]

Department of Clinical Pharmacology, University Hospital Frankfurt, 6000 Frankfurt am Main 70,[1] and Institute for Biomedical and Pharmaceutical Research, 8501 Nürnberg-Heroldsberg,[2] Federal Republic of Germany

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Inhibition of cytochrome P450IA2 activity is an important adverse effect of quinolone antibacterial agents. It results in a prolonged half-life for some drugs that are coadministered with quinolones, such as theophylline. The objective of the study described here was to define the parameters for quantifying the inhibitory potencies of quinolones against cytochrome P450IA2 in vivo and in vitro and to investigate the relationship between the results of both approaches. Cytochrome P450IA2 activity in vitro was measured by using the 3-demethylation rate of caffeine (500 μM) in human liver microsomes. The inhibitory potency of a quinolone in vitro was determined by calculating the decrease in the activity of cytochrome P450IA2 caused by addition of the quinolone (500 μM) into the incubation medium. The mean values (percent reduction of activity without quinolone) were as follows: enoxacin, 74.9%; ciprofloxacin, 70.4%; nalidixic acid, 66.6%; pipemidic acid, 59.3%; norfloxacin, 55.7%; lomefloxacin, 23.4%; pefloxacin, 22.0%; amifloxacin, 21.4%; difloxacin, 21.3%; ofloxacin, 11.8%; temafloxacin, 10.0%; fleroxacin, no effect. The inhibitory potency of a quinolone in vivo was defined by a dose- and bioavailability-normalized parameter calculated from changes of the elimination half-life of theophylline and/or caffeine reported in previously published studies. Taking the pharmacokinetics of the quinolones into account, it was possible to differentiate between substances with and without clinically relevant inhibitory effects by using results of in vitro investigations. The in vitro test described here may help to qualitatively predict the relevant drug interactions between quinolones and methylxanthines that occur during therapy.

The fluoroquinolones have proven to be very effective antibacterial agents (2, 20, 23). Several new agents have been developed over the past decade. Their efficacies in controlling bacterial infections have resulted in the widespread use of these drugs.

Another important biological effect of this drug class concerns the metabolism of several unrelated pharmaceutical agents, resulting in occasional adverse reactions. It has been reported that some of the quinolone antibacterial agents cause a reduced velocity of caffeine and theophylline degradation in vivo (see below) (for a review, see reference 10) and in vitro (14, 40). Furthermore, reductions in the antipyrine metabolism rate and the R-warfarin oxidation rate were observed in humans when quinolones were coadministered with these drugs (23, 24, 46).

In investigations with human liver microsomes, the mechanism of this interaction was determined for ofloxacin, ciprofloxacin, enoxacin, lomefloxacin, and pipemidic acid. A competitive-type inhibition of P450IA2 activity by all of these compounds was found (14). This cytochrome P450 isoform is primarily responsible for the first steps of both caffeine and theophylline metabolism in the liver, as shown in investigations with specific antibodies (5, 37) and genetically engineered cells that express single cytochrome P450 isoforms (13). The main metabolic pathway of caffeine in all systems tested, i.e., 3-demethylation, is mediated almost exclusively by cytochrome P450IA2 (5) and, therefore, may be used as a specific probe for P450IA2 activity (13).

Serious incidents (theophylline intoxication), including death, have occurred because of this drug interaction (3, 25). Thus, the objective of the present study was to define the parameters for quantifying the inhibitory potencies of quinolones against cytochrome P450IA2 in vivo and in vitro and to investigate the relationship between in vitro and in vivo effects. This is of considerable interest in light of the abundance of new quinolones. The probability of encountering these adverse drug reactions should be considered when comparing the therapeutic values of otherwise similar quinolones.

MATERIALS AND METHODS

Quinolones and other chemicals. The following quinolones were supplied by the indicated companies: ofloxacin (Hoechst, Frankfurt, Federal Republic of Germany), lomefloxacin (Searle, Dreieichenhain, Federal Republic of Germany), pipemidic acid (Madaus, Cologne, Federal Republic of Germany), ciprofloxacin (Bayer, Wuppertal, Federal Republic of Germany), enoxacin (Gödecke, Freiburg, Federal Republic of Germany), difloxacin and temafloxacin (Abbott, North Chicago, Ill.), pefloxacin (Rhone-Poulenc, Antony, France), norfloxacin (Merck Sharp & Dohme, Rahway, N.J.), nalidixic acid and amifloxacin (Winthrop, Norderstedt, Federal Republic of Germany), and fleroxacin (Kyorin, Tokyo, Japan).

The following methylxanthines were obtained from the indicated suppliers: caffeine (Serva, Heidelberg, Federal Republic of Germany), 1,7-dimethylxanthine (1,7-DMX; Aldrich, Milwaukee, Wis.), and hydroxypropyltheophylline (Fluka, Buchs, Switzerland). NADP (98%), glucose 6-phosphate, and glucose 6-phosphate-dehydrogenase (from yeast, purity grade II, 140 U/ml) were purchased from Boehringer (Mannheim, Federal Republic of Germany). Acetonitrile, methanol, and tetrahydrofuran were chromatography-grade products from Merck (Darmstadt, Federal Republic of Ger-

* Corresponding author.

many). All other chemicals (analytical grade) were purchased from Merck.

In vitro data. The effect of quinolones on the 3-demethylation of caffeine in human liver microsomes was used to measure the inhibitory activity against cytochrome P450IA2.

(i) Tissue preparation. Donors of liver samples, the preparation of human liver microsomes, and characterization methods were the same as described previously (14).

(ii) Incubations. Incubations were carried out in 0.1 M KH_2PO_4 buffer (pH 7.4), in which all compounds were dissolved. Since the solubilities of some of the quinolones in this buffer were poor, stock solutions of quinolones were prepared by dissolving about 5 μmol in 0.2 ml of 0.1 M NaOH. An adequate volume of this solution (1.5 μmol of inhibitor) was immediately added to buffer containing 0.023 mM glucose 6-phosphate. Together with 0.1 M HCl (volume equal to that of the inhibitor solution), a final volume of 1.5 ml with a final inhibitor concentration of 1 mM was obtained for the stock solutions.

The quinolone to be tested was added to a buffer solution containing about 2 mg of microsomal protein per ml, caffeine, and an NADPH-generating system (for details, see reference 14). The incubation time was 15 min. Caffeine concentrations of 500 μM were chosen to test the inhibitory effect of a quinolone derivative. A quinolone concentration of 500 μM was used. As a control, incubations in the absence of a quinolone but with otherwise identical conditions were carried out. Four independent values were obtained during separate incubations by using human liver microsomes from four donors; each value was derived from duplicate incubations with and without an inhibitor.

The choice of concentrations used in the in vitro test system depended on the analytical conditions. Both substrate and inhibitor concentrations exceeded those that occur in vivo by about 1 order of magnitude. This was necessary because the activity of caffeine metabolism in vitro is low (13), and neither metabolite concentrations nor inhibitory effects could be measured at lower concentrations in vitro. Although it would be possible to extrapolate the effects under chosen in vitro conditions to any substrate and/or inhibitor concentration, such a procedure was not helpful in this study, since the concentrations of both caffeine and the quinolones at the active site of the enzyme were unknown.

(iii) Determination of 1,7-DMX. 1,7-DMX was assayed by high-pressure liquid chromatography (14). 1,7-DMX peak heights relative to those of the internal standard showed a linear correlation ($r > 0.998$) with spiked concentrations in the concentration range from 250 to 100,000 nM. The coefficient of variation of the assay was 7.1% at 250 nM and below 3% at concentrations greater than 1,000 nM. HPLC run times were prolonged by up to 1 h to avoid peak superposition of methylxanthines by the quinolones from a previous run.

(iv) Evaluation of in vitro data. The inhibitory effect ($E_{\text{in vitro}}$) of a compound on microsomes from each donor was determined as cytochrome P450IA2 activity in the presence of this compound relative to that in its absence. This effect is given as a mean ± standard deviation for the four microsome specimens. Inhibitory potency ($P_{\text{in vitro}}$) was calculated as the mean reduction of activity ($P_{\text{in vitro}} = 1 - E_{\text{in vitro}}$).

In vivo data. (i) Inhibitory effects of quinolones. The effects of 10 quinolone antibacterial agents on methylxanthine metabolism in vivo were summarized from previously published data, including our own investigations (4, 7, 9, 11, 16–18, 22, 26, 28, 31, 32, 35, 36, 38, 39, 41–44, 47, 48). Those studies dealt with changes of theophylline and caffeine kinetics caused by the concomitant repeated application of quinolones in therapeutic doses.

(ii) Quinolone pharmacokinetics. The inhibitory effects of the quinolones are expected to be related to their concentrations at the cytochrome P450IA2 site. These concentrations following oral administration are unknown, and even serum quinolone concentrations in interaction studies have been measured only occasionally. The existing data obtained from studies of quinolone pharmacokinetics are not sufficient to enable a reliable estimation of the quinolone concentrations obtained in interaction studies. Thus, only the bioavailability and amount of the quinolone given were taken into account in evaluating inhibitory properties in vivo.

(iii) Evaluation of in vivo data. The extent of inhibition reported in vivo was expressed as follows using mean values for the half-life (or reciprocal clearance): $E_{\text{in vivo}} = 1 -$ (half-life of caffeine or of theophylline when administered alone)/(half-life of caffeine or of theophylline during quinolone coadministration) (equation 1). An in vivo inhibitory effect ($E_{\text{in vivo}}$) of 0 was assumed when a decreased half-life during quinolone coadministration was reported. From these reported effects, we calculated a standard inhibitory potency ($P_{\text{in vivo}}$), which represents a dose- and bioavailability-normalized parameter for each quinolone. To that end, the dose-dependent effects of all compounds were fitted to the log-linear sigmoid dose-effect model described in the following equation by using nonlinear regression analysis (19): $E_{\text{in vivo}} = (b_{\max} \cdot \text{dose}^n)/(\text{ED}_{50}{}^n + \text{dose}^n)$ (equation 2), where $b_{\max}$ is the maximal effect, and ED_{50} is the dose that gives 50% of the maximal effect. The value of $b_{\max}$ used in this equation was 1 (i.e., theoretical prolongation of methylxanthine half-life to infinity); the dose term describes the single-dose size of the drug that was coadministered, which was given once to four times daily (usually twice daily). The exponent n was fixed at 1, because the inclusion of this variable in a nonlinear regression analysis in most cases was not favored by the Akaike model discrimination test (34). All datum points were given the same weight in the analysis.

To allow for differences in quinolone doses in interaction studies, a dose of 1 mmol was chosen for all quinolones to compare the effects between substances. The bioavailability of a quinolone was taken into account by dividing the 1-mmol dose by the absolute oral bioavailability of the substance. For ciprofloxacin a value of 0.60 for oral bioavailability was used (20), for norfloxacin the value was 0.35 (1), and for all other compounds, it was 1.00 (2, 30, 33). Thus, the effect of a dose resulting in a bioavailable drug amount of 1 mmol was calculated from the fitted curves and was used to describe the $P_{\text{in vivo}}$ of a quinolone. These doses (e.g., enoxacin, 321 mg; fleroxacin, 406 mg; ciprofloxacin, 553 mg) are within the range of therapeutic doses for most quinolones (2, 30, 33).

RESULTS

Table 1 presents the $E_{\text{in vitro}}$ values of the quinolones expressed as the remaining activity of P450IA2 for caffeine 3-demethylation when a quinolone (500 μM) was added to the incubation relative to the control activity in the absence of an inhibitor. $P_{\text{in vitro}}$ values are also given in Table 1. The coefficient of variation for $E_{\text{in vitro}}$ between donors was small (about 10%). Enoxacin was the most effective inhibitor of caffeine 3-demethylation tested in vitro, whereas fleroxacin showed no inhibitory activity.

TABLE 1. $P_{in\ vitro}$ of quinolones by using caffeine 3-demethylation in human liver microsomes

Compound	$E_{in\ vitro}$ determined with liver microsomes from four donors (mean ± SD)[a]	$P_{in\ vitro}$[b]
Enoxacin	0.251 ± 0.017	0.749
Ciprofloxacin	0.306 ± 0.067	0.694
Nalidixic acid	0.334 ± 0.056	0.666
Pipemidic acid	0.407 ± 0.028	0.593
Norfloxacin	0.443 ± 0.089	0.557
Lomefloxacin	0.766 ± 0.071	0.234
Pefloxacin	0.780 ± 0.048	0.220
Amifloxacin	0.786 ± 0.038	0.214
Difloxacin	0.787 ± 0.073	0.213
Ofloxacin	0.892 ± 0.099	0.108
Temafloxacin	0.900 ± 0.045	0.100
Fleroxacin	1.044 ± 0.142	0.000

[a] Expressed as the remaining activity of P450IA2 when a quinolone was added to the incubation as a fraction of the control activity.
[b] Relative reduction of enzyme activity.

The $E_{in\ vivo}$ values of the quinolones were assessed from the interaction studies with theophylline or caffeine reported above. The dose-dependent extent of the effects of the quinolones on caffeine metabolism were similar to those on theophylline degradation for all quinolones for which data from interaction studies were available. Whenever any effect of quinolones was found, higher doses caused a greater prolongation of the half-life, resulting in a higher $E_{in\ vivo}$ (Table 2). Although study designs and study subjects differed widely, only small differences between the observed and the calculated effects were found for each quinolone (Table 2). Parameters estimated by using equation 2 for each quinolone are given in Table 3. As an example, the relation between the dose and inhibitory effect in vivo is shown for enoxacin and ciprofloxacin (Fig. 1).

The extent of methylxanthine metabolism inhibition to be expected from the repeated application of a dose that results in a bioavailable quinolone amount of 1 mmol (i.e., 232 to 901 mg) ($P_{in\ vivo}$) was derived from reported values (see above) and is given in Fig. 2. The relationship of inhibitory potency in both test systems is shown by plotting $P_{in\ vivo}$ versus $P_{in\ vitro}$ (Fig. 3). There was no linear relationship present for the substances tested here. However, by separating quinolone groups with high and low inhibitory potencies in vivo and in vitro by mean values, it was shown that pronounced decreases in cytochrome P450IA2 activity ($P_{in\ vitro}$, ≥ 0.39) in microsomes usually correspond to a marked prolongation of the methylxanthine half-life in vivo ($P_{in\ vivo}$, ≥ 0.22) and that minor effects in vitro were related only to slight changes in the pharmacokinetic parameters in volunteers. Exceptions were nalidixic acid, which had a high inhibitory effect in vitro but not in vivo, and pefloxacin, for which the effect in vivo was more pronounced than that in vitro.

DISCUSSION

All quinolones tested (with the exception of fleroxacin) showed an inhibitory effect on caffeine 3-demethylation under the in vitro conditions used in this study. This effect was most pronounced for enoxacin and ciprofloxacin. A possible modification of the in vitro effects because of degradation of the quinolones has been tested for ciprofloxacin (12). Reduction in the concentration of intact ciprofloxacin was not detectable as a result of incubation. The concentration of the M1 metabolite (cleavage of piperazinyl substituent) of ciprofloxacin was less than 0.3% of the ciprofloxacin concentration, and other metabolites were below the limit of detection.

It has been reported that some quinolones (i.e., enoxacin, ciprofloxacin, ofloxacin, lomefloxacin, and pipemidic acid [14, 29]) exert a competitive type of inhibition on caffeine 3-demethylation. The structures of the substances tested previously (14, 29) cover the range of structures of those of the compounds included in the present investigation. Therefore, it is safe to conclude that all congeners used in this study exerted their inhibitory effects by binding to the active site of the enzyme, resulting in a competitive type of inhibition. A competitive inhibition is described by equation 3: $v = (V_{max} \cdot [S])/\{[S] + K_m \cdot (1 + [I]/K_i)\}$, where v is the metabolite formation rate, V_{max} is the maximum metabolite formation rate possible, $[S]$ is the substrate concentration, K_m is the Michaelis-Menten constant, $[I]$ is the inhibitor concentration, and K_i is the inhibitor constant.

By using equation 3, K_i values (or the 50% inhibitory concentration) can be calculated from a single substrate and inhibitor concentration since K_m and V_{max} values have previously been determined for this reaction in the same microsome samples (14). Therefore, the information given by the percent inhibition obtained in the present study is equivalent to that provided by the K_i or 50% inhibitory concentration parameters, which are used more often. However, the percentages were not transformed to avoid a multiplication of the experimental error.

The low standard deviation between donors provides further evidence that P450 isozymes other than P450IA2 (or isoforms coordinately regulated with P450IA2) that are not susceptible to inhibition do not take part in caffeine metabolism (5).

Cytochrome P450IA2 has been reported to be responsible for caffeine 3-demethylation, which is the major caffeine degradation step in humans (5). There is evidence that this isozyme also takes part in 1- and 7-demethylation of caffeine and mediates the main portions of the theophylline demethylation and hydroxylation pathways (although other cytochrome P450 isoforms may also play a minor part in primary theophylline metabolism [37]). Additionally, similar K_m values for all primary metabolic steps of theophylline and caffeine have been reported (6, 14, 15, 21). Thus, it is not surprising that inhibition of P450IA2 activity by the concomitant application of quinolones results in a similar degree of half-life prolongation and/or clearance reduction for both caffeine and theophylline (10). This enables the pooling of in vivo inhibition data for both methylxanthines.

The association between the increasing half-lives of the methylxanthines with higher doses of the inhibitory quinolones (16, 38) may readily be explained by the competitive type of inhibition that has been observed in vitro (14). To quantify the dose dependency of these inhibitory effects, we derived a dose- and bioavailability-normalized inhibitory potency ($P_{in\ vivo}$) for each quinolone for which interaction studies with methylxanthines have been reported previously. This defined a mean increase in the methylxanthine half-life that resulted from repeated, oral coadministration of a quinolone dose representing a bioavailable amount of 1 mmol.

$P_{in\ vivo}$ showed only minor variations within each of the compounds examined in this study, and therefore, it may be a good parameter to use to describe the inhibitory potency of quinolones. Nevertheless, each of the interaction studies

TABLE 2. Effects of quinolone antibacterial agents on methylxanthine elimination in vivo

Quinolone administration		Methylxanthine administration		Effect observed ($E_{in\ vivo}$)[a]	$E_{in\ vivo}$ observed − $E_{in\ vivo}$ estimated	Reference
Compound	Dosing, duration (days)[b]	Compound	Dosing, duration (days)			
Enoxacin	25 mg b.i.d., 7	Theophylline	200 mg, single dose	0.259	0.132	38
Enoxacin	100 mg b.i.d., 7	Theophylline	200 mg, single dose	0.424	0.056	38
Enoxacin	400 mg b.i.d., 7	Theophylline	200 mg, single dose	0.452	−0.247	38
Enoxacin	200 mg b.i.d., 4	Theophylline	4 mg/kg[c], single dose	0.504	−0.034	47
Enoxacin	100 mg b.i.d., 4	Caffeine	230 mg, single dose	0.508	0.140	16
Enoxacin	200 mg b.i.d., 4	Caffeine	230 mg, single dose	0.558	0.020	16
Enoxacin	400 mg b.i.d., 6	Theophylline	300–600 mg b.i.d.	0.614	−0.085	33
Enoxacin	400 mg b.i.d., 7	Theophylline	146 mg b.i.d., 5	0.652	−0.047	28
Enoxacin	500 mg b.i.d., 4	Caffeine	230 mg, single dose	0.720	−0.024	16
Enoxacin	400 mg b.i.d., 4	Caffeine	183 mg o.d., 4	0.835	0.136	26
Pipemidic acid	400 mg b.i.d., 5	Theophylline	260 mg, single dose	0.427	−0.042	44
Pipemidic acid	400 mg b.i.d., 4	Caffeine	230 mg, single dose	0.548	0.079	16
Pipemidic acid	800 mg b.i.d., 1.5	Caffeine	350 mg, single dose	0.600	−0.039	7
Pefloxacin	400 mg b.i.d., 6	Theophylline	300–600 mg b.i.d.	0.291	−0.100	33
Pefloxacin	400 mg b.i.d., 4	Caffeine	183 mg o.d., 4	0.490	0.099	26
Ciprofloxacin	100 mg b.i.d., 4	Caffeine	230 mg, single dose	0.056	−0.005	16
Ciprofloxacin	250 mg b.i.d., 4	Caffeine	230 mg, single dose	0.128	−0.011	16
Ciprofloxacin	500 mg b.i.d., 4	Caffeine	230 mg, single dose	0.209	−0.035	16
Ciprofloxacin	750 mg b.i.d., 7	Theophylline	4 mg/kg, single dose	0.260	−0.066	32
Ciprofloxacin	500 mg b.i.d., 7	Theophylline	125 mg t.i.d., 7	0.266	0.022	36
Ciprofloxacin	750 mg b.i.d., 6	Theophylline	200 mg b.i.d., 20	0.274	−0.052	41
Ciprofloxacin	500 mg b.i.d., 7	Theophylline	5 mg/kg, single dose	0.292	0.048	35
Ciprofloxacin	500 mg b.i.d., 6	Theophylline	300–600 mg b.i.d.	0.310	0.066	33
Ciprofloxacin	750 mg b.i.d., 1.5	Caffeine	100 mg, single dose	0.366	0.040	17
Norfloxacin	400 mg b.i.d., 4	Theophylline	4 mg/kg, single dose	0.084	−0.052	9
Norfloxacin	400 mg b.i.d., 4	Caffeine	230 mg, single dose	0.136	−0.002	16
Norfloxacin	200 mg t.i.d., 3	Theophylline	183 mg, single dose	0.144	0.068	39
Norfloxacin	400 mg b.i.d., 6	Theophylline	4 mg/kg, single dose	0.149	0.008	18
Norfloxacin	800 mg b.i.d., 1.5	Caffeine	350 mg, single dose	0.184	−0.063	7
Norfloxacin	400 mg b.i.d., 7	Theophylline	5 mg/kg, single dose	0.185	0.044	35
Norfloxacin	400 mg b.i.d., 4	Theophylline	142 mg t.i.d., 4	0.207	0.066	4
Fleroxacin	200 mg b.i.d., 5	Theophylline	200 mg b.i.d., 9	0.000	−0.033	43
Fleroxacin	400 mg b.i.d., 5	Theophylline	141 mg b.i.d., 5	0.081	0.017	42
Lomefloxacin	200 mg b.i.d., 5	Theophylline	200 mg b.i.d., 10	0.000	−0.022	22
Lomefloxacin	400 mg b.i.d., 7	Theophylline	125 mg t.i.d., 7	0.018	−0.026	36
Lomefloxacin	400 mg o.d., 5	Theophylline	260 mg, single dose	0.081	0.037	44
Nalidixic acid	500 mg b.i.d., 6	Theophylline	300–600 mg b.i.d.	0.000	−0.048	33
Nalidixic acid	500 mg q.i.d., 7	Theophylline	5 mg/kg, single dose	0.096	0.048	35
Ofloxacin	200 mg b.i.d., 4	Caffeine	230 mg, single dose	0.000	−0.031	16
Ofloxacin	400 mg b.i.d., 6	Theophylline	300–600 mg b.i.d.	0.017	−0.043	33
Ofloxacin	200 mg t.i.d., 3	Theophylline	183 mg, single dose	0.049	0.018	39
Ofloxacin	200 mg b.i.d., 5	Theophylline	200 mg t.i.d., 5	0.052	0.021	11
Ofloxacin	200 mg b.i.d., 4	Theophylline	4 mg/kg, single dose	0.103	0.072	47
Temafloxacin	400 mg b.i.d., 7	Theophylline	146 mg b.i.d., 5	0.086	0.000	28

[a] For calculation of parameters, see text; an $E_{in\ vivo}$ of 0 corresponds to no increase in the methylxanthine half-life during quinolone coadministration; a value of 1 represents a theoretical half-life prolongation to infinity.
[b] o.d., once daily; b.i.d., two times daily; t.i.d., three times daily; q.i.d., four times daily.
[c] Dose per kilogram of body weight.

(see above) showed a high interindividual variation in the $E_{in\ vivo}$ value which may have been due to the well-known intraindividual variation of methylxanthine metabolism in the course of a study and/or to interindividual differences in the disposition of quinolones. Differences in the susceptibilities of individual P450IA2 cytochromes in vivo seem to be of minor importance, since the effects of the quinolones on the enzyme in vitro were very similar between donors (Table 1). Thus, it is possible to estimate mean changes in methylxanthine kinetics because of quinolone coadministration at a defined dose by using equation 2 and the parameters listed in Table 3. For example, coadministration of pefloxacin (200 mg twice daily) in patients with asthma who are taking theophylline is estimated to cause an $E_{in\ vivo}$ of 0.243. This corresponds to a mean prolongation in the theophylline

TABLE 3. Parameters estimated by equation 2 describing the dose-effect relationship of the quinolones and inhibitory activity on methylxanthine elimination in vivo

Compound	ED_{50} (mg)[a]
Enoxacin	172
Ciprofloxacin	1,550
Nalidixic acid	9,917
Pipemidic acid	452
Norfloxacin	2,441
Lomefloxacin	8,760
Pefloxacin	624
Ofloxacin	6,258
Temafloxacin	4,251
Fleroxacin	5,898

[a] The ED_{50} is the oral dose (given twice daily in most cases) estimated to double the caffeine or theophylline half-life when one of the quinolones is coadministered with one of the methylxanthines.

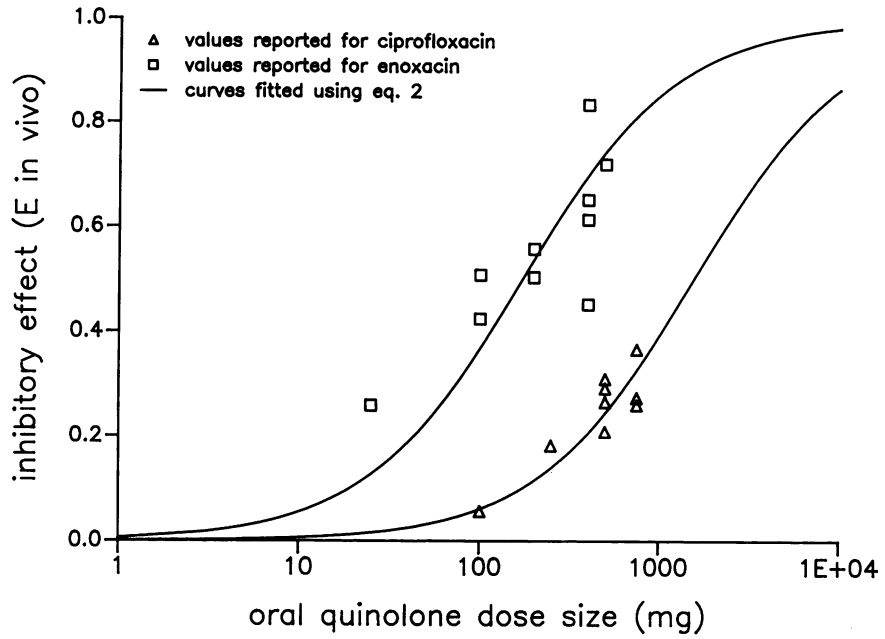

FIG. 1. Influence of enoxacin and of ciprofloxacin dose on P450IA2 activity in vivo. The dose on the x axis describes the size of a single oral quinolone dose which was applied repeatedly. $E_{in\ vivo}$ is 1 minus the quotient of the methylxanthine half-lives reported without and during concomitant quinolone application. 1E + 04, 10^4.

half-life of 32%, which requires a mean dose reduction by the same percentage to maintain steady-state trough levels when an open one-compartment model for theophylline kinetics is applied. However, a corresponding dose adjustment based on mean inhibitory effects in individual patients with long-term theophylline therapy still requires control for possible adverse drug reactions and/or concentrations of drug in serum because of interindividual diversity (see above).

For the majority of quinolones investigated, the inhibitory effects observed under the chosen in vitro conditions were related to qualitatively similar effects in vivo. Enoxacin, pipemidic acid, norfloxacin, and ciprofloxacin caused marked decreases in caffeine metabolite formation rates in vitro ($P_{in\ vitro}$, ≥0.39) and produced a significant prolongation of methylxanthine half-lives in most in vivo studies ($P_{in\ vivo}$, ≥0.22). Temafloxacin, lomefloxacin, ofloxacin, and fleroxacin had only minor effects both in vivo and in vitro.

The inhibitory effects of quinolones on methylxanthine metabolism in vivo are postulated to be the result of a mutually exclusive concurrence at the cytochrome P450IA2

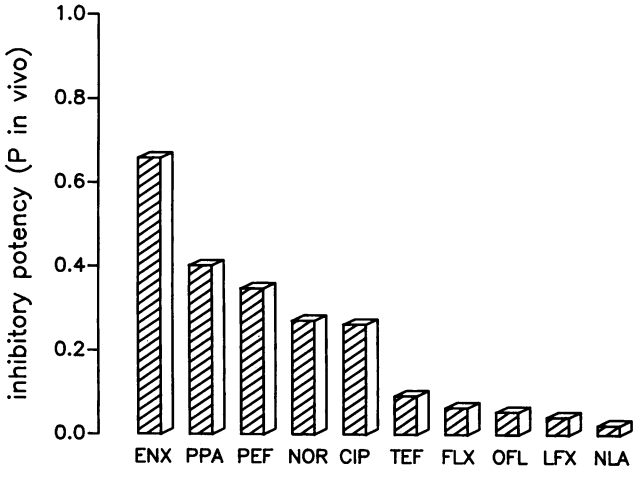

FIG. 2. Inhibitory potency of various quinolones against methylxanthine metabolism in vivo. $P_{in\ vivo}$ is 1 minus the quotient of the methylxanthine half-lives estimated without and during repeated application of a quinolone dose resulting in a bioavailable drug amount of 1 mmol. Abbreviations: ENX, enoxacin; PPA, pipemidic acid; PEF, pefloxacin; NOR, norfloxacin; CIP, ciprofloxacin; TEF, temafloxacin; FLX, fleroxacin; OFL, ofloxacin; LFX, lomefloxacin; NLA, nalidixic acid.

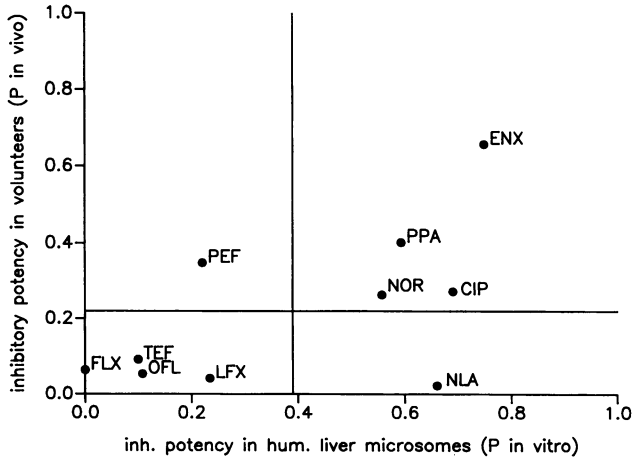

FIG. 3. Relationship of inhibitory potency of quinolones against cytochrome P450IA2 activity in vivo and in vitro. Lines represent mean values that were used to distinguish between substances with minor inhibitory activities in vivo ($P_{in\ vivo}$, <0.22) or in vitro ($P_{in\ vitro}$, <0.39) and those that caused marked levels of inhibition. For definitions of abbreviations, see the legend to Fig. 2.

binding site. The extent of inhibition depends not only on the affinity of a quinolone to the site but also on the concentrations of this drug and possible active metabolites at the cytochrome binding site. This may account for the lack of a complete correlation between in vivo and in vitro data. For example, nalidixic acid showed marked inhibitory activity in vitro, but close to no effects on theophylline pharmacokinetics were reported (35, 48). This drug has pharmacokinetic properties unlike those of all other quinolones tested. The high protein binding of nalidixic acid (more than 90%; 20 to 30% for the other quinolones tested) as well as rapid and extensive metabolism (30) may result in very low concentrations of the drug at the P450IA2 isoform, despite the high doses given in reported methylxanthine interaction studies (up to 500 mg four times daily) (35). Although theophylline metabolism in vivo was not affected by this drug in two studies, a twofold prolongation of antipyrine half-life during nalidixic acid coadministration (1 g four times daily) has been reported previously (23). Antipyrine is metabolized in part by P450IA2, and ciprofloxacin (500 mg twice daily) has been reported to double the elimination half-life of antipyrine in volunteers (24). Thus, the effect of nalidixic acid on P450IA2 activity in vivo requires further evaluation.

Pefloxacin exerts significant inhibitory activity in vivo, occasionally resulting in theophylline intoxication (8), but not in vitro. The reported formation of norfloxacin from pefloxacin, thus producing an active metabolite, may explain this observation. Administration of both pefloxacin or norfloxacin (at identical single doses) results in similar areas under the concentration-time curve for norfloxacin (20). Therefore, norfloxacin contributes to the inhibitory effect of pefloxacin in vivo. Pefloxacin *N*-oxide, which reaches concentrations similar to those of norfloxacin (20), may further augment the extent of inhibition observed in vivo.

Other factors are likely to influence the concentrations of these inhibitors at the binding site in vivo. The poor bioavailabilities of ciprofloxacin and norfloxacin, even when taking into account the amount of substance that reaches the blood after an orally administered dose, may indicate that there is a subsequently reduced penetration of these substances through biological membranes to the cytochrome P450IA2.

By extrapolating our in vitro results for amifloxacin and difloxacin to the inhibitory effects on methylxanthine metabolism in vivo, we predict that a clinically relevant prolongation of the caffeine or theophylline half-life will not be observed during coadministration of these quinolone antibacterial agents. During the review process for the manuscript, sparfloxacin became available for in vitro tests. It reduced caffeine 3-demethylation in vitro by 38.8%, which was still within the range for quinolones with minor in vitro effects. In fact, this quinolone had no effect on the theophylline half-life in one in vivo interaction study (27) and reduced theophylline clearance by only 9% (mean) in a trial in patients with asthma (45). The corresponding $P_{\text{in vivo}}$ that was estimated by assuming complete oral bioavailability was 0.037.

Results of the present study indicate that knowledge both of the inhibitory activity of a quinolone (and probably those of its major metabolites) in the in vitro model described here and of the pharmacokinetics (metabolites, protein binding, and bioavailability) of the substance enables a qualitative estimation of its inhibitory properties on cytochrome P450IA2 activity and, thus, on the elimination of methylxanthine from volunteers and patients.

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REFERENCES

1. **Adhami, Z. N., R. Wise, D. Weston, and B. Crump.** 1984. The pharmacokinetics and tissue penetration of norfloxacin. J. Antimicrob. Chemother. **13:**87–92.
2. **Andriole, V. T. (ed.).** 1988. The quinolones. Academic Press Ltd., London.
3. **Bem, J. L., and R. D. Mann.** 1988. Danger of interaction between ciprofloxacin and theophylline. Br. Med. J. **296:**1131.
4. **Bowles, S. K., Z. Popovski, M. J. Rybak, H. B. Beckman, and D. J. Edwards.** 1988. Effect of norfloxacin on theophylline pharmacokinetics at steady state. Antimicrob. Agents Chemother. **32:**510–512.
5. **Butler, M. A., M. Iwasaki, F. P. Guengerich, and F. K. Kadlubar.** 1989. Human cytochrome $P450_{PA}$ (P-450IA2), the phenacetin O-deethylase, is primarily responsible for the hepatic 3-demethylation of caffeine and N-oxidation of carcinogenic arylamines. Proc. Natl. Acad. Sci. USA **86:**7696–7700.
6. **Campbell, M. E., M. D. Grant, T. Inaba, and W. Kalow.** 1987. Biotransformation of caffeine, paraxanthine, theophylline, and theobromine by polycyclic aromatic hydrocarbon-inducible cytochrome(s) P-450 in human liver microsomes. Drug Metab. Dispos. **15:**237–249.
7. **Carbo, M. L., J. Segura, R. de la Torre, J. M. Badenas, and J. Cami.** 1989. Effect of quinolones on caffeine disposition. Clin. Pharmacol. Ther. **45:**234–240.
8. **Conri, C., M. C. Larhgue, L. Abs, M. C. Mesire, M. P. Vincent, F. Haramburu, and J. Constans.** 1990. Convulsions chez une malade traitee par pefloxacine et theophylline. Therapie **45:**358.
9. **Davis, R. L., H. W. Kelly, R. W. Quenzer, J. Standefer, B. Steinberg, and J. Gallegos.** 1989. Effect of norfloxacin on theophylline metabolism. Antimicrob. Agents Chemother. **33:**212–214.
10. **Edwards, D. J., S. K. Bowles, C. K. Svenson, and M. J. Rybak.** 1988. Inhibition of drug metabolism by quinolone antibiotics. Clin. Pharmacokinet. **15:**194–203.
11. **Fourtillan, J. B., J. Granier, B. Saint-Salvi, J. Salmon, A. Surjus, D. Tremblay, M. Vincent Du Laurier, and S. Beck.** 1986. Pharmacokinetics of ofloxacin and theophylline alone and in combination. Infection **14**(Suppl. 1):S67–S69.
12. **Fuhr, U., D. Beermann, and A. H. Staib.** 1989. Unpublished data.
13. **Fuhr, U., J. Doehmer, N. Battula, C. Wölfel, C. Kudla, Y. Keita, and A. H. Staib.** 1992. Biotransformation of caffeine and theophylline in mammalian cell lines genetically engineered for expression of single cytochrome P450 isoforms. Biochem. Pharmacol. **43:**225–235.
14. **Fuhr, U., T. Wolff, S. Harder, P. Schymanski, and A. H. Staib.** 1990. Quinolone inhibition of cytochrome P-450-dependent caffeine metabolism in human liver microsomes. Drug Metab. Dispos. **18:**1005–1010.
15. **Grant, D. M., M. E. Campbell, B. K. Tang, and W. Kalow.** 1987. Biotransformation of caffeine by microsomes from human liver. Biochem. Pharmacol. **36:**1251–1260.
16. **Harder, S., A. H. Staib, C. Beer, A. Papenburg, W. Stille, and P. M. Shah.** 1988. 4-Quinolones inhibit biotransformation of caffeine. Eur. J. Clin. Pharmacol. **35:**651–656.
17. **Healy, D. P., R. E. Polk, L. Kanawati, D. T. Rock, and M. L. Mooney.** 1989. Interaction between oral ciprofloxacin and caffeine in volunteers. Antimicrob. Agents Chemother. **33:**474–478.
18. **Ho, G., M. G. Tierny, and R. E. Dales.** 1988. Evaluation of the effect of norfloxacin on the pharmacokinetics of theophylline. Clin. Pharmacol. Ther. **44:**35–38.
19. **Holford, N. H. G., and L. B. Sheiner.** 1981. Unterstanding the dose-effect relationship. Clin. Pharmacokinet. **6:**429–453.
20. **Jaehde, U.** 1989. Charakterisierung von Transport- und Eliminationsvorgängen neuerer Gyrase-Hemmer im menschlichen Organismus. Ph.D. thesis. Freie Universität, Berlin.
21. **Kalow, W., and M. Campbell.** 1988. Biotransformation of caf-

feine by microsomes. ISI Atlas Sci. Pharmacol. **1**:381–386.
22. **Kuzuya, T., K. Takagi, R. Apichartpichean, I. Muraoka, M. Nadai, and T. Hasegawa.** 1989. Kinetic interaction between theophylline and a newly developed quinolone, NY-198. J. Pharmacobio-Dyn. **12**:405–409.
23. **Ludwig, E., H. Graber, T. Perenyi, and L. Benesch.** 1975. Examinations of drug interactions with nalidixic and oxolinic acids. Orv. Hetil. **116**:2947–2948.
24. **Ludwig, E., E. Szekely, A. Csiba, and H. Graber.** 1988. The effect of ciprofloxacin on antipyrine metabolism. J. Antimicrob. Chemother. **22**:61–67.
25. **Maesen, F. P. V., J. P. Teengs, C. Baur, and B. I. Davies.** 1984. Quinolones and raised plasma concentrations of theophylline. Lancet **ii**:530.
26. **Mahr, G., R. Seelmann, J. Dammeyer, M. Seibel, P. Muth, B. Gottschalk, U. Stephan, and F. Sörgel.** 1990. The effect of pefloxacin and enoxacin on the elimination of caffeine in human volunteers. Program Abstr. 3rd Int. Symp. New Quinolones, abstr. 425.
27. **Mahr, G., R. Seelmann, B. Gottschalk, U. Stephan, and F. Sörgel.** 1990. Program Abstr. 30th Intersci. Conf. Antimicrob. Agents Chemother., abstr. 1259.
28. **Mahr, G., R. Seelmann, R. Granneman, J. Sylvester, B. Gottschalk, C. Jürgens, P. Muth, U. Stephan, and F. Sörgel.** 1989. Program Abstr. 29th Intersci. Conf. Antimicrob. Agents Chemother., abstr. 216.
29. **Mizuki, Y., M. Kamaura, T. Yamaguchi, Y. Sekine, and M. Hashimoto.** 1989. Interaction of enoxacin with theophylline in rats. Arzneim.-Forsch. **39**:593–597.
30. **Neuman, M.** 1988. Clinical pharmacokinetics of the newer antibacterial 4-quinolones. Clin. Pharmacokinet. **14**:96–121.
31. **Niki, Y., R. Soejima, H. Kawane, M. Sumi, and S. Umeki.** 1987. New synthetic quinolone antibacterial agents and serum concentration of theophylline. Chest **92**:663–669.
32. **Nix, D. E., J. M. DeVito, M. A. Whitbread, and J. J. Schentag.** 1987. Effect of multiple dose oral ciprofloxacin on the pharmacokinetics of theophylline and in docyanine green. J. Antimicrob. Chemother. **19**:263–269.
33. **Nix, D. E., and J. J. Schentag.** 1988. The quinolones. An overview and comparative appraisal of their pharmacokinetics and pharmacodynamics. J. Clin. Pharmacol. **28**:169–178.
34. **Perrella, F. W.** 1988. EZ-FIT: a practical curve-fitting microcomputer program for the analysis of enzyme kinetic data on IBM-PC compatible computers. Anal. Biochem. **174**:437–447.
35. **Prince, R. A., E. Casabar, C. G. Adair, D. B. Wexler, J. Lettieri, and J. E. Kasik.** 1989. Effect of quinolone antimicrobials on theophylline pharmacokinetics. J. Clin. Phamacol. **29**:650–654.
36. **Robson, R. A., E. J. Begg, H. C. Atkinson, D. A. Saunders, and C. M. Frampton.** 1990. Comparative effects of ciprofloxacin and lomefloxacin on the oxadative metabolism of theophylline. Br. J. Clin. Pharmacol. **29**:491–493.
37. **Robson, R. A., J. O. Miners, A. P. Matthews, I. Stupans, D. Meller, M. McManus, and D. J. Birkett.** 1988. Characterisation of theophylline metabolism by human liver microsomes. Inhibition and immunochemical studies. Biochem. Pharmacol. **37**: 1651–1659.
38. **Rogge, M. C., W. R. Solomon, A. J. Sedman, P. G. Welling, R. D. Toothaker, and J. G. Wagner.** 1988. The theophylline-enoxacin interaction. I. Effect of enoxacin dose size on theophylline disposition. Clin. Pharmacol. Ther. **44**:579–587.
39. **Sano, M., I. Yamamoto, J. Ueda, E. Yoshikawa, H. Yamashina, and M. Goto.** 1987. Comparative pharmacokinetics of theophylline following two fluoroquinolones co-administration. Eur. J. Clin. Pharmacol. **32**:431–432.
40. **Sarkar, M., R. E. Polk, P. S. Guzelian, C. Hunt, and H. T. Karnes.** 1990. In vitro effect of fluoroquinolones on theophylline metabolism in human liver microsomes. Antimicrob. Agents Chemother. **34**:594–599.
41. **Schwartz, J., L. Jauregui, J. Lettieri, and K. Bachmann.** 1988. Impact of ciprofloxacin on theophylline clearance and steady-state concentrations in serum. Antimicrob. Agents Chemother. **32**:75–77.
42. **Seelmann, R., G. Mahr, B. Gottschalk, U. Stephan, and F. Sörgel.** 1989. Influence of fleroxacin on the pharmacokinetics of theophylline. Rev. Infect. Dis. **11**(Suppl. 5):S1100.
43. **Soejima, R., Y. Niki, and M. Sumi.** 1989. Effect of fleroxacin on serum concentrations of theophylline. Rev. Infect. Dis. **11**(Suppl. 5):S1099.
44. **Staib, A. H., S. Harder, U. Fuhr, and C. Wack.** 1989. Interaction of quinolones with the theophylline metabolism in man: investigations with lomefloxacin and pipemidic acid. Int. J. Clin. Pharmacol. Ther. Toxicol. **27**:289–293.
45. **Takagi, K., K. Yamaki, M. Nadai, T. Kuzuya, and T. Hasegawa.** 1991. Effects of a new quinolone, sparfloxacin, on the pharmacokinetics of theophylline in asthmatic patients. Antimicrob. Agents Chemother. **35**:1137–1141.
46. **Toon, S., K. J. Hopkins, F. M. Garstang, L. Aarons, A. Sedman, and M. Rowland.** 1987. Enoxacin-warfarin interaction: pharmacokinetic and stereochemical aspects. Clin. Pharmacol. Ther. **42**:33–41.
47. **Wijnands, W. J. A., T. J. Janssen, P. J. M. Guelen, T. B. Vree, and T. M. C. de Witte.** 1988. The influence of ofloxacin and enoxacin on the metabolic pathways of theophylline in healthy volunteers. Pharm. Weekbl. (Sci.) **10**:272–276.
48. **Wijnands, W. J. A., T. B. Vree, and C. L. A. van Herwaarden.** 1986. The influence of quinolone derivatives on theophylline clearance. Br. J. Clin. Pharmacol. **22**:677–683.