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Effect of Antacid on Absorption of the Quinolone Lomefloxacin

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The effect of antacid on the absorption of lomefloxacin (LFLX) in humans was studied. When LFLX was orally administered concomitantly with aluminum- and magnesium-containing antacids under fasting conditions, its level in plasma decreased by one-half and its area under the concentration-time curve was reduced by 40% compared with the levels observed after treatment with LFLX alone. The urinary recovery value also decreased by 40%. No such effects were noted after coadministration of LFLX and a nonmetallic antacid. This study confirmed the existence of chelate complexes of LFLX with Al^{3+} and Mg^{2+} and examined the chelating strength. The stability constants of LFLX with Al^{3+} and Mg^{2+} were measured and compared with those of ofloxacin and norfloxacin; little difference was observed among them. LFLX was found to bind more strongly with Al^{3+} than with Mg^{2+}. Further, the existence of chelate formation was proven by ^{13}C-nuclear magnetic resonance spectroscopy. The decrease in the LFLX level in plasma in humans could be explained by a reduced absorption of the Al^{3+}- and Mg^{2+}-LFLX chelate complexes.

Lomefloxacin (LFLX) is a well-absorbed fluoroquinolone with a strong activity against gram-positive and -negative organisms (3, 13). Recently, it has been reported that the ciprofloxacin level in plasma after oral administration of a single dose of ciprofloxacin decreases markedly when a metallic antacid containing aluminum and magnesium is given concomitantly (4, 10, 14). Similar observations for humans have been reported for LFLX (15) and ofloxacin (OFLX) (11). The decrease may occur because of reduction of drug absorption by interaction with metal ions to produce a chelated form. Little has been reported on the chelate formation of these quinolones with metals in a metallic antacid. In view of the therapeutic importance of these drugs, we conducted this study to determine the effect of the metal ion on the level in plasma by administering LFLX together with the aluminum- and magnesium-containing drug Kolantyl and the nonmetallic antacid Ulgut.

We studied the pharmacokinetics of LFLX after administration of LFLX alone and with Kolantyl at clinical dose levels (LFLX, 200 mg; Kolantyl, 2 g) and with Ulgut (400 mg). Kolantyl and Ulgut are popular gastrointestinal drugs used widely in Japan. Since aluminum hydroxide gel alone has been found to lower the absorption of LFLX (15), similar effects of metal components, aluminum hydroxide, and magnesium oxide have been anticipated. Ulgut was chosen as a nonmetallic gastrointestinal agent, and its effect was compared with that of Kolantyl. We found that LFLX produced a metal chelate with stability constants of 7 and 2.8 for Al^{3+} and Mg^{2+}, respectively. The chelation phenomena of OFLX and norfloxacin (NFLX) with Al^{3+} and Mg^{2+} were also examined and compared with that of LFLX. We discuss the effect of Al^{3+} and Mg^{2+} on the absorption of LFLX in humans on the basis of the chelation equilibrium of LFLX with Al^{3+} and Mg^{2+}.

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MATERIALS AND METHODS

Drugs. The LFLX capsule is a pharmaceutical preparation consisting of 200 mg of LFLX hydrochloride. One dose of granules of the antacid Kolantyl (Shionogi & Co., Ltd.) contained 400 mg of dried aluminum hydroxide gel, 200 mg of magnesium oxide, and 5 mg of dicyclomine hydrochloride. The capsule of the nonmetallic antacid Ulgut (Shionogi & Co., Ltd.) contained 200 mg of benexate hydrochloride β-cyclodextrin clathrate. The OFLX and NFLX were obtained as Tarivid tablets (Daiichi Seiyaku Co. Ltd.) and Baccidal tablets (Kyorin Seiyaku Co. Ltd.), respectively. The drugs OFLX and NFLX were extracted from Tarivid and Baccidal tablets and purified. The tablets were powdered and extracted with solvent. The chloroform extract of Tarivid, after being filtered, was evaporated to dryness. The residue was dissolved with dichloromethane-ethanol (1:1) and cooled. The deposited crystals were dried under vacuum. The elemental analysis and the spectral data (nuclear magnetic resonance [NMR], infrared, and UV) were consistent with the structure of OFLX. NFLX was extracted with dichloromethane and then evaporated to dryness. The residue was dissolved with dichloromethane and, after ethanol was added (1:1), was cooled. The deposited crystals were dried for 7 h at 100°C. The elemental analysis and the spectral data (NMR, infrared, and UV) were consistent with the structure.

Pharmacokinetics in humans. Six Japanese male volunteers, aged 25 to 46 years (mean age, 35.2 years) and weighing 48 to 68 kg (mean weight, 60.0 kg) were enrolled for this study. Each volunteer gave informed written consent for the study. They were divided to three groups of two each, and each subject was assigned to three dose regimens, including a single capsule of LFLX alone, LFLX with 2 g of Kolantyl granules, and LFLX with two Ulgut capsules, according to the Latin square design, with a 1-week interval between administrations. Subjects fasted from 10 p.m. the night before until 5 h after receiving the LFLX the next morning. The drugs were taken with 100 ml of tap water.

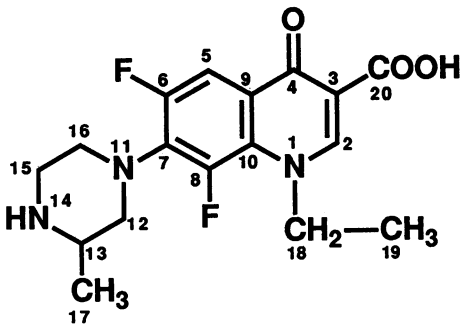

FIG. 1. LFLX.

About 3 ml of blood sample was withdrawn from each volunteer into heparinized tubes before (0 h) and at 0.5, 1, 1.5, 2, 3, 4, 6, 8, 12, and 24 h after dosing and immediately centrifuged for 15 min. Urine samples were collected before dosing (0 h) and then in block samples at 0 to 2, 2 to 4, 4 to 6, 6 to 8, 8 to 12, and 12 to 24 h after dosing. Both plasma and urine samples were stored at −20°C until they were analyzed.

The effects of the coadministered drugs, Kolantyl and Ulgut, on the absorption of LFLX were statistically evaluated by using an analysis of variance at $P = 0.05$.

High-performance liquid chromatography (HPLC) method. LFLX levels in plasma and urine samples were determined by a method reported previously (12).

The assay method was validated with the performance data. The standard solutions were prepared with the blank human plasma (CELLect, ICN Biomedicals, Inc., Irvine, Calif.) and urine samples from the volunteer spiked with LFLX. The calibration curves for both assays were reproducible, with the linearity of regression coefficient being more than 0.999 and the slope of the straight line being close between days ($n = 5$). The quantitation limit of the assay was 0.01 μg/ml for the plasma sample and 0.05 μg/ml for the urine sample. In the plasma assay, three solutions with concentrations of 0.1, 0.8, and 3.1 μg/ml, which covered the LFLX level after the administration to humans, were measured repeatedly. In the urine assay, three solutions with concentrations of 20, 200, and 710 μg/ml were measured. In the within-day plasma assay, the coefficients of variation at the three concentrations were 3.1, 1.6, and 1.7%, respectively, and the coefficient values for urine assay were 3.3, 2.3, and 1.0%, respectively. The between-day precision was examined by replicate measurements ($n = 5$) by using the same samples with the above three concentrations. The coefficients of variation were 6.6, 1.4, and 1.8%, respectively, for the plasma assay and 4.3, 1.7, and 2.1%, respectively, for the urine assay. The precision at the quantitation limit varied 6.5% for the plasma assay and 7.5% for the urine assay.

Bioassay method. LFLX concentrations in plasma and urine samples were measured by a method reported previously (12).

By using the cylinder-plate method with *Escherichia coli* NIHJ JC-2 as the test organism and Mueller-Hinton agar (Difco Laboratories, Detroit, Mich.) as the assay medium, the diameter of the inhibitory zone due to LFLX's antibacterial activity was measured. LFLX in the standard solutions, with eight concentration levels which ranged from 0.1 to 12.5 μg/ml, was assayed repeatedly. The inhibitory diameters, which were measured five times for each concentration, were averaged. A linear relationship between the logarithmic value of the mean diameter and the drug concentration was obtained. The correlation coefficient ranged from 0.998 to 0.9996. Little difference in coefficient value was observed between the plasma standard solution (Consera Nissui as a control serum; Nissui Seiyaku Co. Ltd., Tokyo, Japan) and the urine standard solution (pH 7.0 phosphate buffer), being more than 0.998. The quantitation limit was 0.1 μg/ml. The precision was evaluated by five replicate measurements with the solutions of 0.1, 0.8, and 3.1 μg/ml. The within-day coefficients of variation were 1.3, 1.4, and 1.5% in the plasma standard and 2.7, 1.3, and 1.1% in the urine standard (for both, $n = 5$). The between-day coefficients of variation (5 days) for the above concentrations of each standard solution were 6.1, 5.2, and 2.9% and 1.6, 0.9, and 1.6%, respectively.

The band-culture method (7) used for the investigation of metal ion effect was assessed by assaying aqueous solutions of LFLX (0.5 to 8 μg/ml). *E. coli* 7437 as the test organism and trypto-soy agar (pH 7.0; Eiken Chemical Co. Ltd., Tokyo, Japan) as the assay medium were used. The quantitation limit was 0.5 μg/ml. Very little deviation was found between the observed value and the predicted value from the standard curve, with the coefficient of variation being greater than 0.998. The precision was evaluated by four replicate measurements with 0.5- and 8.0-μg/ml solutions. The within-day coefficients of variation for the two solutions were 1.3 and 2.1%, respectively. The between-day coefficients of variation for the two solutions were 2.1% and 3.0%, respectively.

According to our data for the method validation, both the HPLC and the bioassay method determined LFLX in biological samples accurately, with the same level of precision and reproducibility, and were sufficient to be used for pharmacokinetic study.

Measurement of the stability constant. A potentiometric method was used for measurement of the stability constant of the chelates. Drugs (0.1 to 0.3 mmol) were dissolved in water (40 ml), and the pH of each solution was adjusted to a pH close to that of the metal solutions by adding trace amounts of 0.1 M HCl and NaOH. Then, 1 M NaCl (5 ml) was added to each solution and the solutions were diluted to 50 ml with water. Forty milliliters each of the drug solutions was pipetted into a thermostated cell equipped with a jacket and titrated at 25°C with the metal solutions (15 mM) in 0.1 M NaCl by using a Comtite-101 titrator (Hiranuma Sangyo Co. Ltd.) equipped with a glass-Ag/AgCl electrode.

Measurement of ^{13}C-NMR spectra of the chelates. ^{13}C-NMR spectral data were recorded with a Varian XL-400 spectrometer at 100.577 MHz. The spectrum of LFLX hydrochloride was measured in D_2O ($2.0\text{-}6.5 \times 10^{-2}$ M). An equimolar amount of $AlCl_3 \cdot 6H_2O$ or $MgCl_2 \cdot 6H_2O$ and one-half the LFLX concentration was added to the LFLX solution and measured. Typical measurement conditions were as follows: spectral width, 21008.4 Hz; acquisition time, 0.679 s; pulse flip angle, 30°; pulse delay time, 0.250 s; transient number of acquisition pulse, 52,224. ^{13}C-chemical shifts (δ_c) were obtained from acetonitrile as an internal standard (CH_3CN; δ_c, 1.71 ppm).

RESULTS

Assayed values in the presence of metal ions. The aqueous solution of LFLX (0.5 to 2.0 μg/ml) containing Al^{3+} or Mg^{2+} (0.1 to 10 mM) was assayed by HPLC, and the concentration of LFLX was compared with those obtained without metal ions. The HPLC method determined the concentration of

TABLE 1. Mean pharmacokinetic parameters for LFLX after oral administration of a single dose of LFLX (200 mg) alone or with the antacid Kolantyl or Ulgut[a]

Study	Addition	C_{max}[b] (μg/ml)	T_{max}[b] (h)	AUC[c] (μg · h/ml)	$t_{1/2}(\beta)$	Urinary recovery (% of dose)
1	None	1.91 ± 0.55	1.6 ± 0.7	12.64 ± 2.09	6.83 ± 1.88	67.5 ± 9.0
2	Ulgut (400 mg)	1.96 ± 0.41	1.3 ± 0.5	12.43 ± 1.58	7.54 ± 1.84	67.3 ± 5.9
3	Kolantyl (2 g)	1.03 ± 0.50	1.4 ± 1.0	7.48 ± 1.05	7.67 ± 1.75	41.2 ± 13.3

[a] Data are means ± standard deviations (n = 6).
[b] Observed value.
[c] Calculated by trapezoidal rule from 0 to 24 h.

LFLX without being affected by the coexistent Al^{3+} or Mg^{2+}. On the contrary, the assayed values determined by the bioassay method decreased under the influence of the metal ions. Levels of LFLX the same as when assayed alone (in the range of 1 to 8 μg/ml) were recorded in the presence of 0.1 mM Al^{3+} or 1 mM Mg^{2+}, but the levels dropped as the concentration of the metal ion was increased from 1 to 100 mM. The values at 1 and 10 mM Al^{3+} were 60 to 70% and 20 to 30%, respectively. In the case of 10 and 100 mM Mg^{2+}, the concentration decreased to 80 to 90%.

Pharmacokinetic analysis. Semilogarithmic plots of LFLX concentrations showed that LFLX eliminated biexponentially in the postabsorptive phase. Levels of LFLX in plasma were fitted to a two-compartment model with a first-order absorption process by a nonlinear least-squares method by using the NONLIN program. Each concentration value was weighed equally. Elimination half-life ($t_{1/2}\beta$) was calculated by using the elimination rate constant (β) in the elimination phase estimated by the pharmacokinetic analysis. The area under the concentration-time curve (AUC) from 0 to 24 h was calculated by trapezoidal rule. The maximum concentration of drug in plasma (C_{max}), AUC, and urinary recovery after dosing with Kolantyl were significantly lower than those after the other two dose regimens. The power of detection of difference was sufficient for AUC and urinary recovery, but not for C_{max}. On the other hand, there were no differences in time to C_{max} (T_{max}) and $t_{1/2}\beta$. The pharmacokinetic parameters are shown in Table 1.

Stability constant of the chelates. The chelation equilibria are formed stepwise between the ligand quinolone and metal ion, as follows: $M + L = ML$, $K_1 = [ML]/[M][L]$; $ML + L = ML_2$, $K_2 = [ML_2]/[ML][L]$; $ML_2 + L = ML_3$, $K_3 = [ML_3]/[ML_2][L]$. The stability constants, K_1, K_2, and K_3, were measured by the potentiometric method established by Bjerrum (1). Titration of metal ion in the aqueous solution of the quinolones resulted in an increase in the hydrogen ion released from the carboxylic acid group to which the metal ion binds. The pH change with increasing metal ion was analyzed according to Bjerrum's method, and the stability constants, K_1, K_2, and K_3, were determined by using the two observed values, the average of the coordination number and the concentration of the free ligand (L). In the chelation with Mg^{2+}, since the average of the coordination number was observed to be less than 2, K_1 and K_2 were calculated. Table 2 shows the stability constants (logarithmic value) of LFLX, OFLX, and NFLX for Al^{3+} and Mg^{2+}.

TABLE 2. Stability constants of LFLX, OFLX, and NFLX with Al^{3+} and Mg^{2+}

Drug	Metal ion	log K_1	log K_2	log K_3
LFLX	Al^{3+}	7.12	5.47	4.71
	Mg^{2+}	2.80	2.14	—[a]
OFLX	Al^{3+}	7.13	5.40	5.34
	Mg^{2+}	2.82	2.66	—
NFLX	Al^{3+}	7.03	5.44	5.45
	Mg^{2+}	2.93	2.65	—

[a] —, Little or no equilibration occurs.

^{13}C-NMR spectra of the chelates. ^{13}C-NMR spectra of LFLX were measured in D_2O in the absence and presence of metal ion. Figures 2 and 3 illustrate the chemical shifts of LFLX and its chelate with Al^{3+} and Mg^{2+}, respectively. The signals which shifted markedly or resulted in broadening by the metal chelation were picked up: C-2, C-3, C-4, and C-20 in LFLX (Fig. 1). The two spectra of the solutions containing LFLX and the metal ion in ratios of 1:1 and 2:1 were compared with that of LFLX alone.

DISCUSSION

Figure 4 shows the mean LFLX levels in plasma after oral administration, and the curves were fitted to a two-compartment model. The levels of LFLX in plasma after coadministration with Kolantyl were significantly lower than those of LFLX alone, while LFLX after coadministration with Ulgut showed the same high levels in plasma as LFLX alone. The T_{max} ranged from 0.5 to 3 h, but there was no significant difference in the mean T_{max}s of each regimen. The C_{max} after coadministration with Kolantyl was about one-half and the AUC was 40% less than those observed after treatment with LFLX alone. There were significant differences in C_{max}s and AUCs between the administrations of LFLX alone and with Kolantyl, but there were no significant differences between those of administrations of LFLX alone and with Ulgut. Little or no differences in $t_{1/2}\beta$ were observed among the three regimens. These pharmacokinetic parameters calculated from the HPLC data agreed well with those from the bioassay method.

Mean urinary recovery for 24 h after oral administration of LFLX alone was 67.5%, while those after coadministration with Ulgut and Kolantyl were 67.3 and 41.2%, respectively. Kolantyl significantly decreased the urinary recovery by 40%, which corresponds to the reduced LFLX levels in plasma.

The results show that the nonmetallic antacid Ulgut had no effect on the absorption of LFLX and its whole pharmacokinetics but that metallic antacid Kolantyl affected the absorption of LFLX by reducing its bioavailability by 40%. The $t_{1/2}\beta$ of LFLX was not affected by Kolantyl. These results show that metallic antacid Kolantyl affects only the absorption of LFLX. It is reasonable to assume that the metallic antacid Kolantyl forms a chelate with LFLX in the gastrointestinal tract, leading to reduced absorption.

In order to confirm the validity of the assay values, the effect of Al^{3+} and Mg^{2+} on the HPLC and the bioassay

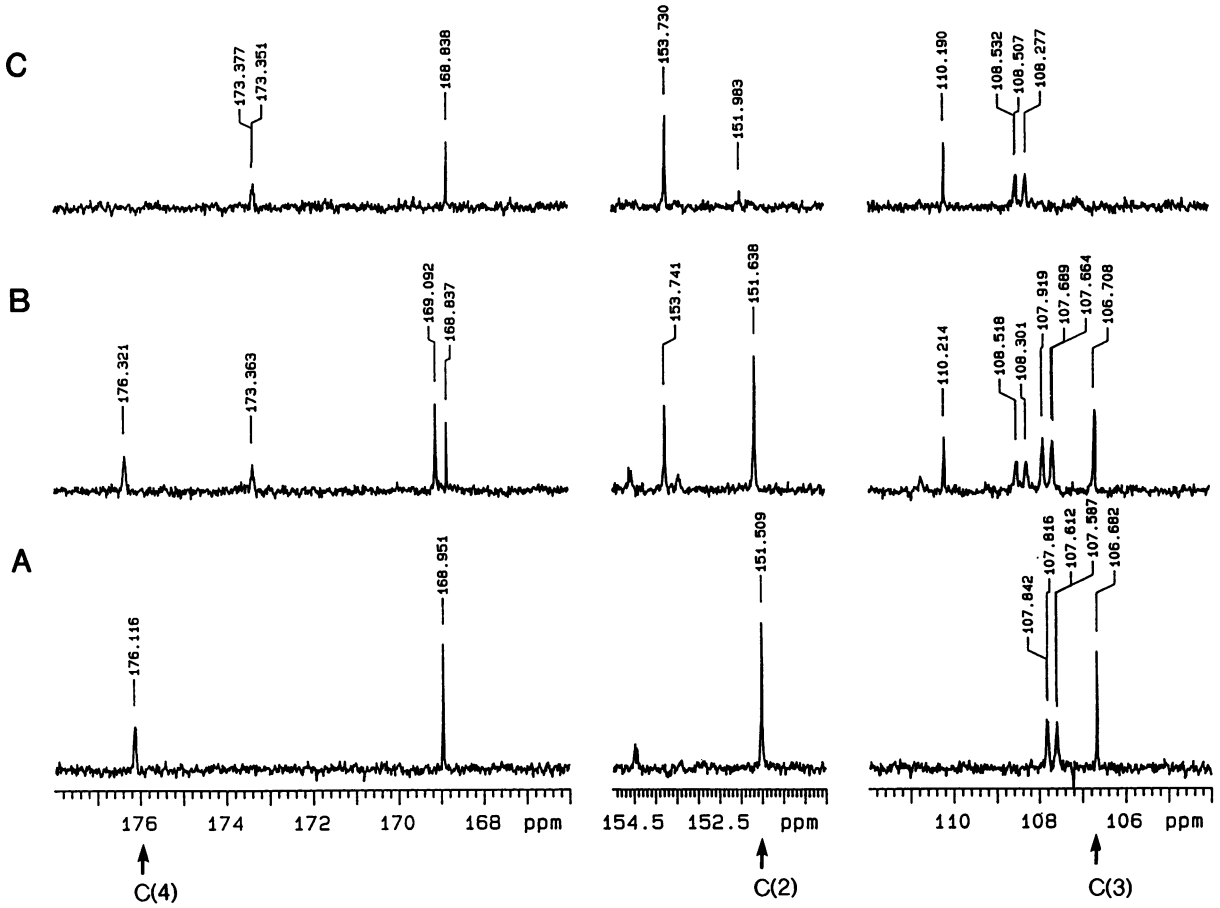

FIG. 2. ^{13}C-NMR spectra of LFLX hydrochloride in D_2O in the absence and presence of Al^{3+}. (A) LFLX hydrochloride. (B) LFLX hydrochloride with Al^{3+} (2:1). (C) LFLX hydrochloride with an equimolar amount of Al^{3+}

method was investigated. Since the chelation of nalidixic acid with various metals (log K_1, 1 to 6) has been observed (16), LFLX at the 10^{-5} level may be expected to form chelate with the metal ions containing 0.1 to 10 mM. The HPLC results showed no effect of the metals on the assay values, suggesting that it determines the amount of LFLX chelate as the apparent free LFLX, i.e., the HPLC method seems to give an estimate of the total amount of free LFLX and its chelated form. The bioassay method, on the other hand, was affected by the excess amount of metal ion; the assay values of 1 to 8 μg/ml of sample were lowered significantly in the presence of Al^{3+}, more than 1 mM, and were lowered in the presence of Mg^{2+} more than 10 mM. These findings indicate that the bioassay method does not determine the amount of chelate compound but does determine that of the LFLX alone when it exists as a mixture of the free and the chelated forms.

The values obtained by the bioassay method were compared with those found by the HPLC method. The data were obtained from the study of healthy volunteers to whom LFLX and Kolantyl were administered concomitantly. LFLX levels in both plasma and urine measured by the HPLC method correlated well with those found by the bioassay method, as shown in Fig. 5. This agreement means that both HPLC and bioassay methods determine the amount of free LFLX and that the decrease in the level of LFLX in plasma by 40% was not due to an effect of the metal ion on the determinations.

Chelation phenomena are known to occur between nalidixic acid and Al^{3+} (9, 16) and between ciprofloxacin and Fe^{3+} (6), but no stability constants for these metal ions have been measured. Table 2 shows that the binding of LFLX was much stronger to Al^{3+} than to Mg^{2+}. The K_1 in the Al^{3+} binding was larger than the K_2. The K_1 for Mg^{2+} binding was close to the K_2. The table also shows the stability constants of OFLX and NFLX, which were measured by the same method. The binding strength for Al^{3+} was at the same level for the three quinolones. In the Mg^{2+} binding measurement NFLX was bound slightly more strongly than LFLX or OFLX.

Metal chelation affects the resonance of carbons to induce shifts in their ^{13}C-NMR spectra, and this was used to reveal the chelate formation of nalidixic acid with Cu^{2+}. It has been shown that when Cu^{2+} binds to a ligand, the carbons near the binding site are relaxed rapidly by the paramagnetic ions and their signals are broadened (2). Al^{3+} binding induces diamagnetic shifts for the carbon signals (5). Mendoza-Diaz and Pannell (8) demonstrated the change of the chemical shifts of the carbons near the binding site of the compound with Cu^{2+} (COOH group and the carbonyl group). A difference in the ^{13}C-chemical shifts for LFLX has been observed in the presence and absence of Al^{3+} and Mg^{2+}, suggesting chelate formation. In this experiment, the effect of metal ion on the chemical shift was examined to ascertain chelation. In a solution containing Al^{3+} and LFLX in a ratio of 1:1, in which a large amount of LFLX binds (99.89%, calculated

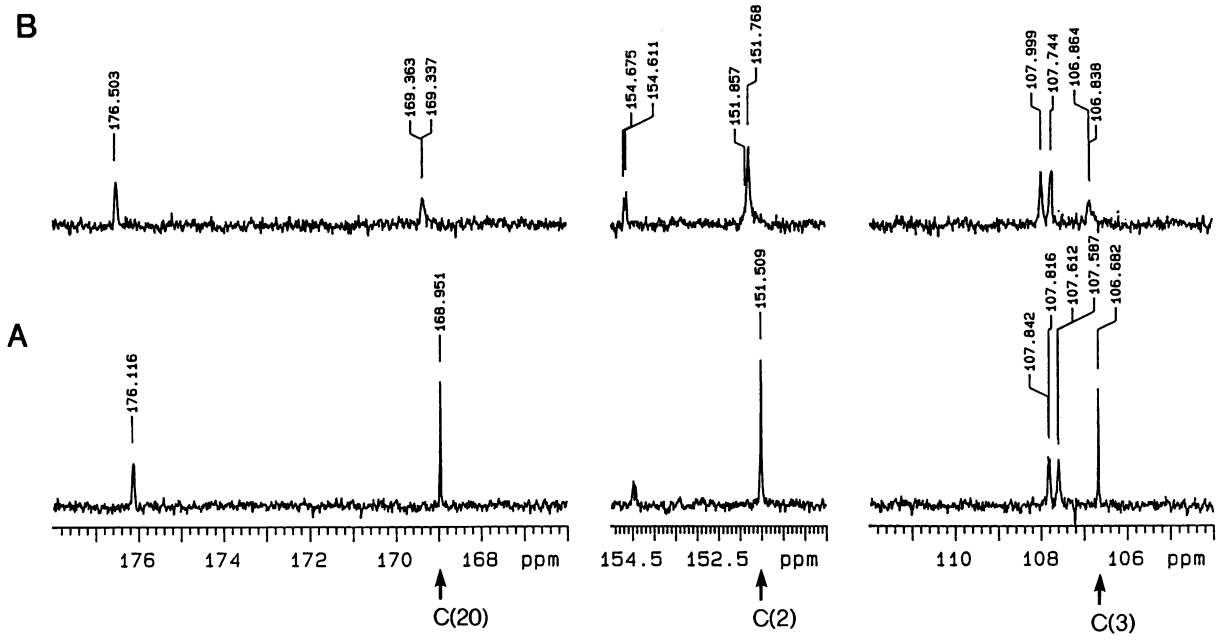

FIG. 3. ^{13}C-NMR spectra of LFLX hydrochloride in D_2O in the absence and presence of Mg^{2+}. (A) LFLX hydrochloride. (B) LFLX hydrochloride with an equimolar amount of Mg^{2+}.

from the K_1) to Al^{3+}, the signals at C-2 and C-3 shifted downfield from 151.51 to 153.74 ppm and 106.68 to 110.19 ppm, respectively (compare Fig. 2C and A). C-4 showed an upfield shift from 176.12 to 173.35 ppm. Much smaller shifts, with most of them being downfield (0 to 1 ppm as $\Delta\delta$), were observed for other carbons. In a mixed solution containing half the amount of Al^{3+} (2:1), in which free LFLX and its chelate (49.99%, calculated from the K_1) existed in the same ratio, chemical shifts of both the free form and the chelate were simultaneously observed, as shown in Fig. 2B. In the same experiment with Mg^{2+}, addition of an equimolar amount of Mg^{2+} resulted in broadening of the signals of C-2, C-3, and C-20, which are shown as the peaks at δ_c 151.51, 106.68, and 168.95, respectively (Fig. 3). Smaller shifts, most of them being downfield (0 to 0.5 ppm as $\Delta\delta$), were observed for other carbons. When less Mg^{2+} (ligand/metal; 2:1) was added, there was little or no change in the chemical shifts compared with the 1:1 spectra, which is different from the case of the Al chelate. Allowing the chemical shifts to change and broaden in ^{13}C-NMR spectrum indicates the occurrence of the chelation of LFLX with Al^{3+} and Mg^{2+}. The signal of the carbonyl carbon (C-4) and those of the two carbons (C-2 and C-3) adjacent to the carboxyl group shifted more largely with Al^{3+} than those of the other carbons, and the signals of the carboxyl carbon (C-20) and the neighboring carbons (C-2 and C-3) were broadened with Mg^{2+}, suggesting the metal binding with the carboxyl and carbonyl groups.

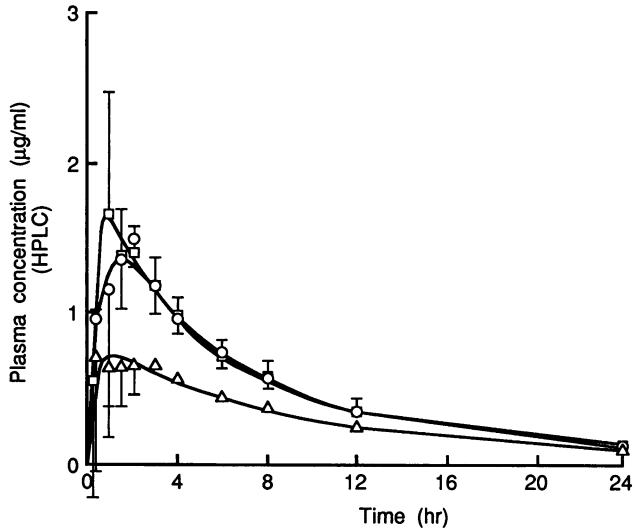

FIG. 4. Mean concentration of LFLX in plasma in volunteers after oral administration of 200 mg alone or with Ulgut (400 mg) or Kolantyl (2 g). Symbols: ○, alone; □, with 400 mg of Ulgut; △, with 2 g of Kolantyl.

The experiment with a mixture of the free LFLX and the chelate (1:1 and 2:1 ligand/metal solutions) showed that the rate of the ligand exchange of the Al^{3+} chelate is slow on the NMR time scale and that the exchange rate of ligand of the Mg^{2+} chelate is fast, suggesting a slower chelation rate for Al^{3+} to LFLX than for Mg^{2+}. In fact, time was needed for Al chelation to reach a constant pH (ca. 2 min) by exchanging Al^{3+} for H^+ of the carboxylic acid on the titrimetry described above.

LFLX undergoes chelation with metals. The chelation equilibria and the change of the ^{13}C-NMR spectra demonstrated the existence of the metal-LFLX complex. Aluminum and magnesium in the antacid Kolantyl are transformed to Al^{3+} and Mg^{2+} in acidic gastric juice. When LFLX and the antacid are administered concomitantly in humans, LFLX forms chelates with the excess metal ions (20-fold). The chelation seems to occur mainly with Al^{3+}, which manifests stronger binding strength than Mg^{2+}. The LFLX level in plasma seems to decrease after coadministration with the two metals. The metal-chelate formation was responsible for the diminished absorption of LFLX in the

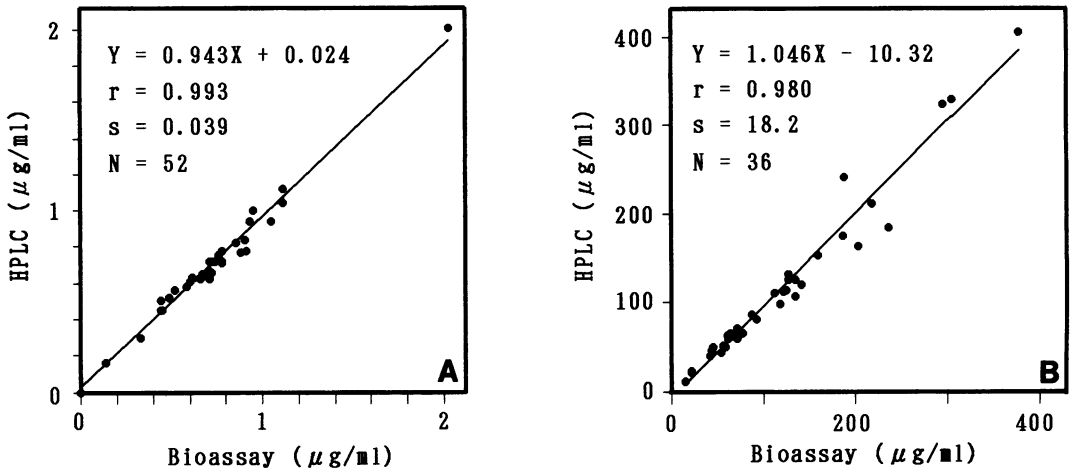

FIG. 5. Correlation between HPLC and bioassay analysis of LFLX hydrochloride in plasma (A) and urine (B). Abbreviations: r, regression coefficient; s, standard deviation; N, number of data.

gastrointestinal tract, which led to a reduction in LFLX levels in plasma.

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