Comparison of Antibacterial Activity of Fluoroquinolones with Their Sucralfate-complexes against Clinically-isolated Bacteria

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Oral fluoroquinolones are widely known to form chelate complexes with metal-containing drugs, resulting in inhibition of their intestinal absorption. However, for intestinal sterilization, the concomitant regimen may be a selective and effective strategy due to decreased absorption of fluoroquinolones result in the retainment of antibiotics at the intestine if the mixture still perpetuated antibacterial activity. Therefore, to clarify whether the mixture of fluoroquinolones and sucralfate affects their antibacterial activity or not, we conducted *in vitro* study. According from the checkerboard study using a microdilution method with Mueller-Hinton broth, the antibacterial activity of these fluoroquinolones-sucralfate mixtures equaled to the parent fluoroquinolones even in the presence of sucralfate at the molar ratio of [sucralfate : fluoroquinolone] was less than 166, and the minimal inhibitory concentrations for clinical isolated *Escherichia coli* and *Pseudomonas aeruginosa* strains were independent of the existence of sucralfate. These data imply that the chelated forms of each fluoroquinolone retain antibacterial activity even in the presence of the recommended therapeutic doses of sucralfate in clinical practice.

Key words —— fluoroquinolone, interaction, antibacterial activity, chelate, sucralfate

INTRODUCTION

Oral fluoroquinolones are commonly administered not only conventional infection diseases caused by microorganisms such as respiratory and urinary tract infections but also prophylaxis use for treatment of endogenous infection in patients undergoing allogeneic hematopoietic stem cell transplantation (allo-HSCT).[1–4] Because the endogenous infection induced by Gram-negative bacteria is a clinically significant problem in compromised hosts such as allo-HSCT recipients,[5,6] and selective intestinal sterilization is a key strategy.[7]

Fluoroquinolones generally form stable metal chelate complexes if these were concomitantly administered with drugs containing metal cations such as aluminum or magnesium, which cause inhibition of their intestinal absorption[8] and following suppression of blood fluoroquinolone concentrations. This phenomenon means that high concentration of fluoroquinolone-cation complexes remain in the intestine. Therefore, the concomitant regimen would be a selective and effective strategy for digestive decontamination if antibacterial activity of fluoroquinolones is maintained even in the chelated forms. However, reported antibiotic activity of various fluoroquinolones with metal complexes is controversial. Some described that the reduction of antibacterial activity was observed,[9–11] and others were not.[12]

Therefore, to provide a uniform criterion for commingling usage of fluoroquinolones with sucralfate, we attempted a comparison of antibacterial activity of fluoroquinolones with their sucralfate-complexes against clinically-isolated bacteria by *in vitro* study. Here we choose, sucralfate as a metal-containing drug because it has the most intensive interaction property against fluoroquinolones in comparison with other metal cations.[8,10,11]

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MATERIALS AND METHODS

Chemicals —— Ciprofloxacin (CPFX), lomefloxacin (LFLX), ulifloxacin (PUFX), and tosufloxacin (TFLX) were provided courtesy of Bayer AG (Leverkusen, Germany), Shionogi & Co. Ltd. (Osaka, Japan), Meiji Ltd. (Tokyo, Japan), Taishotoyama Pharmaceutical Co. Ltd. (Tokyo, Japan), respectively. Enoxacin (ENX) and sparfloxacin (SPFX) were provided courtesy of Dainippon Sumitomo Pharma Co. Ltd. (Osaka, Japan). Gatifloxacin (GFLX) and norfloxacin (NFLX) were provided courtesy of Kyorin Co. Ltd. (Tokyo, Japan). Levofloxacin (LVFX) and ofloxacin (OFLX) were provided courtesy of Daiichi Sankyo Co. Ltd. (Tokyo, Japan). Ulifloxacin is an activated metabolite of prulifloxacin (PUFX). Sucralfate was provided courtesy of Chugai Co. Ltd. (Tokyo, Japan). Each fluoroquinolone powder was dissolved in sterile distilled water followed by dropwise addition of phosphoric acid until dissolved. Subsequent dilution was prepared in sterile distilled water. The sucralfate powder was suspended in sterile distilled water. Other reagents were of the highest grade commercially available.

Bacterial Strains and Culture Medium —— *Escherichia coli* (*E. coli*) strain ATCC25922 was employed as the test organism in this study. *E. coli* strains 1 to 8 (clinical isolates) and *Pseudomonas aeruginosa* (*P. aeruginosa*) strains 1 to 39 (clinical isolates) were kindly supplied by the staff of the Department of Clinical Laboratory, Kagawa University Hospital (Kagawa, Japan). Mueller-Hinton broth (MHB), (pH 7.4) which was purchased from Becton, Dickinson & Co., (Franklin Lakes, NJ, U.S.A.) was employed for the determination of the minimal inhibitory concentration (MIC) in serial dilution tests.

Antibacterial Activity —— An overnight culture of test organism at 35°C in MHB was diluted with saline to yield an initial inoculum of approximately 10^6 colony forming units (CFU)/ml in each well. The MICs of ten fluoroquinolones against *E. coli* strain ATCC25922 was defined as the lowest antibiotic concentration inhibiting visible bacterial growth after overnight incubation at 35°C by turbidity, and each MIC was adopted to the corresponding initial fluoroquinolone concentration for the following experiments. The effect of sucralfate at the concentration range from 0.063 to 4 mg/ml was determined by a two-way full checkerboard design on a 96-well microplate using a two-fold dilution scheme. Antibacterial activity of each sample was defined as the visible bacterial growth after overnight incubation at 35°C by turbidity. The mixture of sucralfate and each fluoroquinolone was incubated for 1 hr in a shaking incubator at a room temperature before applying to the plate. The incubation period for 1 hr was chosen because Tanaka *et al.*[13] reported that the chelation of fluoroquinolones with sucralfate was achieved within 1 hr. The supernatant from fluoroquinolone-sucralfate suspension was obtained by centrifugation at 20000 × *g* for 30 min at room temperature.

Statistical Analysis —— MICs of fluoroquinolones alone and in combination with sucralfate against clinical isolated strains were compared using Student's *t*-test and Welch's *t*-test, as appropriate, respectively. All *p*-values were two-tailed, and $p < 0.05$ was considered significant.

RESULTS AND DISCUSSION

Fluoroquinolones are widely used to treat not only conventional infection diseases but also intestinal sterilization. Meanwhile, fluoroquinolones are prohibited to concomitant administration with drugs that contain aluminum, calcium, or magnesium at the same time as fluoroquinolones due to the dramatic reduction in the oral bioavailability of them by co-administration of metal cations.[8] When viewed from different directions, this phenomenon means that high concentration of fluoroquinolone-cation complexes remain in the intestine, and if the complexes could posses their antibacterial activity, the concomitant regimen would be a selective and effective strategy or, at least, unconcerned way for digestive decontamination. However, there is a little information whether the chelated forms of fluoroquinolones have antibacterial activity or not. Some reports said that the concomitant use of fluoroquinolones and cation-containing drugs caused the reduction of antibacterial activity,[9–11] and others were not.[12] Therefore, to clarify the degree for the decrease of antibacterial activity of fluoroquinolones by aluminum-containing antacid, we conducted the growth-inhibitory experiments of ten fluoroquinolone against *E. coli* strain ATCC25922 in MHB in the presence or absence of sucralfate (Table 1).

Sucralfate was marketed in Japan in 1968 as an antiulcer agent, and that stimulates the defense

Table 1. Effect of Sucralfate on Antibacterial Activity of Ten Fluoroquinolones Towards *E. coli* Strain ATCC25922

Sucralfate	CPFX (0.008 μg/ml*)		Molar ratio of	ENX (0.063 μg/ml*)		Molar ratio of
(mg/ml)	Mixture	Supernatant	sucralfate : CPFX	Mixture	Supernatant	sucralfate : ENX
0	+	+		+	+	
0.063	+	−	1456	+	−	166
0.125	+	−	2889	−	−	330
0.25	+	−	5778	−	−	661
0.5	+	−	11556	−	−	1321
1	−	−	23112	−	−	2642
2	−	−	46224	−	−	5284
4	−	−	92447	−	−	10569
Sucralfate	GFLX (0.016 μg/ml*)		Molar ratio of	LFLX (0.063 μg/ml*)		Molar ratio of
(mg/ml)	Mixture	Supernatant	sucralfate : GFLX	Mixture	Supernatant	sucralfate : LFLX
0	+	+		+	+	
0.063	+	−	759	+	−	186
0.125	+	−	1507	−	−	369
0.25	+	−	3013	−	−	737
0.5	+	−	6027	−	−	1475
1	−	−	12053	−	−	2950
2	−	−	24106	−	−	5900
4	−	−	48212	−	−	11800
Sucralfate	LVFX (0.016 μg/ml*)		Molar ratio of	NFLX (0.063 μg/ml*)		Molar ratio of
(mg/ml)	Mixture	Supernatant	sucralfate : LVFX	Mixture	Supernatant	sucralfate : NFLX
0	+	+		+	+	
0.063	+	−	699	+	−	153
0.125	+	−	1387	+	−	304
0.25	+	−	2773	+	−	607
0.5	−	−	5547	−	−	1215
1	−	−	11093	−	−	2429
2	−	−	22187	−	−	4858
4	−	−	44374	−	−	9716
Sucralfate	OFLX (0.031 μg/ml*)		Molar ratio of	PUFX (0.008 μg/ml*)		Molar ratio of
(mg/ml)	Mixture	Supernatant	sucralfate : OFLX	Mixture	Supernatant	sucralfate : PUFX
0	+	+		+	+	
0.063	+	−	352	+	−	1742
0.125	+	−	698	+	−	3455
0.25	−	−	1397	−	−	6911
0.5	−	−	2793	−	−	13821
1	−	−	5586	−	−	27643
2	−	−	11173	−	−	55286
4	−	−	22346	−	−	110572
Sucralfate	SPFX (0.008 μg/ml*)		Molar ratio of	TFLX (0.008 μg/ml*)		Molar ratio of
(mg/ml)	Mixture	Supernatant	sucralfate : SPFX	Mixture	Supernatant	sucralfate : TFLX
0	+	+		+	+	
0.063	+	−	1481	+	−	2244
0.125	+	−	2938	+	−	4452
0.25	+	−	5877	+	−	8904
0.5	−	−	11753	+	−	17808
1	−	−	23507	+	−	35616
2	−	−	47013	−	−	71232
4	−	−	94026	−	−	142464

Antibacterial activity of each sample is denoted as follows: +, positive activity; −, negative activity. *The initial concentrations of each fluoroquinolone in MHB were the MIC against *E. coli* strain ATCC25922 (values shown in parentheses).

mechanisms of the mucosa.[14] Sucralfate possesses 16 aluminum atoms in one molecule, and the solubility was less than 1% at pH 6.[15] If 1% of sucralfate was dissolved, at least 4.83 μM of aluminum would be present in the reaction mixture when the sucralfate concentration was 0.063 mg/ml. By the way, complexation reactions between aluminum and several fluoroquinolones have been studied, and it was found that the formation of the stable complexes with fluoroquinolone to aluminum ratios from [1 : 1] to [3 : 1].[16] In this study, the initial concentration of each fluoroquinolone in MHB was determined from the MIC against *E. coli* strain ATCC25922, and it varied from 0.013 (0.008 μg/ml; TFLX) to 0.197 (0.063 μg/ml; NFLX) μM (Table 1). Considering that the free aluminum concentration was expected to 4.83 μM from 0.063 mg/ml sucralfate, all fluoroquinolones studied here could make a complex with dissolved aluminum even at the minimum concentration of sucralfate. It was reported that some fluoroquinolones-aluminum complexes lowered their parent antibacterial activity.[17] However, as shown in Table 1, inhibitory effect of sucralfate on antibacterial activity of studied ten fluoroquinolones were not observed when sucralfate concentration was 0.063 mg/ml, indicating that the fluoroquinolone-aluminum complex shows antibacterial activity comparable to that of free fluoroquinolones. Interestingly, antibacterial activity was disappeared with the increase of sucralfate (Table 1). TFLX was less subject to sucralfate (1 mg/ml) followed the rank order CPFX = GFLX > LVFX = NFLX = SPFX > OFLX = PUFX > ENX = LFLX. If fluoroquinolones-aluminum complexes were the critical compounds for antibacterial activity, it would not be affected by sucralfate concentration because sufficient (at least 25 times of aluminum presents in 0.063 mg/ml sucralfate and 0.197 μM NFLX) aluminum was dissolved even at the minimum concentration of sucralfate, and the concentration of fluoroquinolones were constant. According from these results, it was expected that adsorption reaction between fluoroquinolones and sucralfate would exist. In addition, supernatant of each reaction mixture gave no antibacterial activity (Table 1). Taking into account the results of these experiments, it may be concluded that sucralfate adsorbs not only free fluoroquinolones as described[13] but also fluoroquinolone-aluminum complex under our experimental conditions, and that high concentration of sucralfate (molar ratio of [sucralfate : fluoroquinolone]; 11556 < for CPFX, 166 < for ENX, 6027 < for GFLX, 186 < LFLX, 2773 < for LVFX, 607 < for NFLX, 698 < for OFLX, 3455 < for PUFX, 5877 < for SPFX, and 35616 < for TFLX) inhibits antibacterial activity of parent fluoroquinolones due probably to the increased adsorption.

Next, in order to ascertain whether the sucralfate-fluoroquinolone mixture exerts effective antibacterial activity against clinical isolated pathogens or not, we applied two fluoroquinolones (NFLX and CPFX) in combination with or without sucralfate to clinical isolated *E. coli* and *P. aeruginosa* strains. In this study, the sucralfate concentration was kept constant (0.063 mg/ml), and the molar ratio of [sucralfate : NFLX] was prepared from 0.02 (512 μg/ml) to 77 (0.125 μg/ml) and [sucralfate : CPFX] was from 0.09 (128 μg/ml) to 728 (0.016 μg/ml) by varying the concentration of these fluoroquinolones. Our preliminary experiments by high-performance liquid chromatography[18] indicate that these fluoroquinolones would be present as the fluoroquinolones-aluminum complexes in the

Table 2. Antibacterial Activity of NFLX and CPFX Alone and in Combination with Sucralfate Against Clinical Isolated Strains

Organism	Drugs	MIC (μg/ml)			*p*-value
		Range	MIC_{50}	MIC_{90}	
E. coli (*n* = 8)	NFLX alone	0.125–512	256	333	1.00
	NFLX with sucralfate	0.125–512	256	333	
	CPFX alone	0.016–128	64	128	0.88
	CPFX with sucralfate	0.016–128	48	128	
P. aeruginosa (*n* = 39)	NFLX alone	0.125–256	0.5	2.4	0.95
	NFLX with sucralfate	0.125–256	0.5	3.2	
	CPFX alone	0.031– 32	0.25	1.2	0.65
	CPFX with sucralfate	0.031– 64	0.25	1.2	

MIC_{50}/MIC_{90}, MIC at which 50% and 90% of the isolates are inhibited, respectively. The concentration of concomitant sucralfate was 0.063 mg/ml in each well.

mixture due to the dramatic reduction of the parent fluoroquinolone concentration (data not shown). As shown in Table 2, MIC values showed no significant differences between fluoroquinolone alone and fluoroquinolone with sucralfate for two clinical isolated pathogens, because the molar ratios of these [sucralfate : fluoroquinolones] were below the effective values (607 for NFLX and 11556 for CPFX, respectively). According to the information provided in the drug package insert of NFLX, CPFX, and sucralfate, the maximum daily dose is 800 mg (2.51 mmol), 600 mg (1.56 mmol), and 3 g (1.44 mmol) respectively, and the ratio of [sucralfate : NFLX] and [sucralfate : CPFX] are 0.57 and 0.92, which are far below the critical ratio for [sucralfate : NFLX (607)] and [sucralfate : CPFX (11556)]. Taking into account the our results, the combination therapy of sucralfate with, at least, NFLX and CPFX seems to have less affect in the antibacterial activity of these fluoroquinolones under ordinary medical practice except for the absorption processes.[19] It was reported that aluminum hydroxide possessed adsorbent properties for antibiotics including LVFX, OFLX, ENX, and NFLX,[13] which result in the decrease in bioavailability of these drugs.[20] However, there is no information about the antibacterial activity of these adsorbed compounds.

In conclusion, our present data suggests that sucralfate can interact not only by chelate-forming property by dissolved aluminum but also by adsorption reactions, and the chelated forms of each fluoroquinolone retain antibacterial activity even in the presence of the recommended therapeutic doses of sucralfate in clinical practice. At present, some oral aminoglycoside antibiotics are applied for intestinal sterilization such as vancomycin, tobramycin,[21] and neomycin[22] because these drugs are nonabsorbable antibiotics. However, special attention would be paid to issues of ototoxicity, renal insufficiency, and vestibular toxicity in case of large amount of dosage or mucosal lesions are present. In 2007, we had reported the preventive effects of fluoroquinolones such as CPFX or NFLX for endogenous infection in patients receiving various allo-HSCT.[2] Judging from our present result, it seems to be effective strategy to co-administrate fluoroquinolone and sucralfate in order to maintain the fluoroquinolone retention time in the intestine and decrease the absorption into the bloodstream without affect the parent antibiotic activities of fluoroquinolones for intestine sterilization. Further studies are needed to clarify whether this hypothesis is useful or meaningless attempt.

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