Mutagenicity Studies of Five 4-Quinolone Antibiotics in *Salmonella, Drosophila* and Cultured Chinese Hamster Lung (CHL) Cells

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Abstract – 4-Quinolone antibiotics (pefloxacin, ciprofloxacin, norfloxacin, ofloxacin and enoxacin) were tested for mutagenicity in *Salmonella typhimurium* TA98, TA100, TA1535, TA1537, TA1538 and TA102, for chromosomal aberrations in cultured Chinese hamster lung (CHL) cells, and for wing somatic mutations and recombinations (wing spot) in *Drosophila*. Five 4-quinolones did not show any mutagenicity in *Salmonella typhimurium* TA98, TA100, TA1535, TA1537 and TA1538. However, they were mutagenic in *Salmonella typhimurium* TA102 with and without metabolic activation in both plate incorporation method and preincubation method. Ciprofloxacin induced structural chromosome aberrations in CHL cells both with and without metabolic activation, and the frequencies were 6% and up to 28%, respectively. Pefloxacin showed equivocal evidence, however, norfloxacin, ofloxacin and enoxacin did not induce the structural chromosome aberrations both in the presence and absence of metabolic activation. In the wing spot assay in *Drosophila*, ofloxacin increased the frequency of small single spots significantly in a dose-dependent manner but there was no dose-dependent increase of single or twin spots in the others.

Keywords □ 4-Quinolone antibiotics, Mutagenicity, *Salmonella typhimurium*, *Drosophila*, Chinese hamster lung cells

Pefloxacin, ciprofloxacin, norfloxacin, ofloxacin and enoxacin are 4-quinolone antibiotics (Fig. 1), and the fluorinated piperazinyl-substituted quinolone derivatives are increasingly prescribed for the treatment of various bacterial infections. Compared with the earlier quinolone, nalidixic acid, the newer 4-quinolones have a wider antibacterial spectrum including *Pseudomonas aeruginosa* and some Gram positive cocci, and they show improved pharmacokinetic properties (Hooper and Wolfson, 1985; Wolfson and Hooper, 1985). It is thought that all 4-quinolones exert their antibacterial activities via inhibition of DNA gyrase, an essential type II DNA topoisomerase (Gellert, 1981; Shen *et al.*, 1985; Wolfson and Hooper, 1985; Piddock *et al.*, 1987).

The genotoxicity of these drugs have been studied by many investigators using various test systems (Philips, 1987; Philips *et al.*, 1987; Piddock *et al.*, 1987; Holden *et al.*, 1989; Gocke, 1991; Fort, 1992; Power and Phillips, 1993; Mamber *et al.*, 1993; Albertini *et al.*, 1995). However, the mutagenic difference of 4-quinolones between plate incorporation method and preincubation method with and without metabolic activation system in *Salmonella typhimurium* TA102 have not been reported. Chromosome aberration test of 4-quinolones has not been con-

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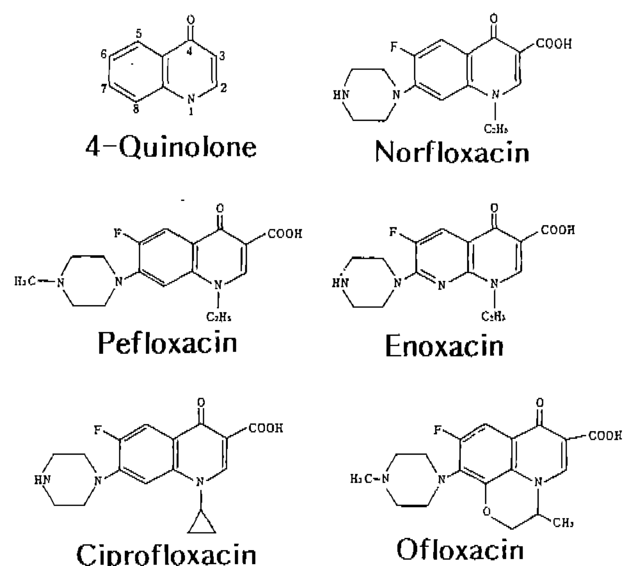

Fig. 1. Structure of 4-quinolone antibiotics.

ducted thoroughly with and without metabolic activation system, some discrepancies still exist between results, and no report is available on *Drosophila* mutagenicity.

Therefore, in this paper we studied the mutagenic potential of 4-quinolone antibiotics in short-term baterry system using *Salmonella typhimurium* TA 102 with and without metabolic activation system both in plate incorporation and preincubation methods, and chromosomal aberrations in cultured Chinese hamster lung cells and wing somatic mutations and recombinations in *Drosophila*. We also confirmed the absence of mutagenicity of these durgs in *Salmonella typhimurium* TA98, TA100, TA1535, TA1537 and TA1538.

MATERIALS AND METHODS

Antibiotics

4-Quinolone derivatives were obtained as follows: pefloxacin (as methane sulfonate) from Rhône-poulenc Korea Pharmaceutical Ltd.; ciprofloxacin (as hydrochloride) from Bayer Korea Pharmaceutical Ltd.; norfloxacin from Sam Jin Pharmaceutical Ltd.; ofloxacin from Cheil Pharmaceutical Ltd.; enoxacin from Dong-A Pharmaceutical Ltd.

Salmonella mutation assay

Bacterial strains

Salmonella typhimurium strains TA98, TA100, TA 1535, TA1537, TA1538 and TA102 were obtained from Dr. Bruce Ames, University of California, Berkeley, U.S. A. The strains were maintained and checked for the genotype as described by Maron and Ames (1983).

Chemicals and doses

The stock solutions of norfloxacin, ofloxacin and enoxacin were prepared by dissolving in 0.02N-NaOH or in distilled water to 1 mg/ml. They were filtered (0.2 μm, Nalgene) and diluted to the desired concentration with sterilized distilled water. Six doses were chosen so that the high doses exhibited some degree of toxicity. The positive control compounds were 2-aminoanthracene, mitomycin-C and sodium azide (Sigma); 2-nitrofluorene (Aldrich); Mutagen ICR-191 (Polysciences). All mutagens were dissolved in dimethylsulfoxide, except mitomycin-C and sodium azide in distilled water.

S9 fraction and S9-mix

S9 fraction was prepared from the liver of Aroclor 1254 (Alltech) induced Sprague-Dawley male rats, and stored as described by Maron and Ames (1983). S9-mix was prepared immediately before each assay and S9-mix contained 4% of S9 fraction.

Mutagenicity assay

All chemicals were tested using the preincubation procedures of the *Salmonella* assay (Maron and Ames, 1983; Haworth *et al.*, 1983; Zeiger *et al.*, 1988). Plate incorporation method also was used for strain TA102 (Maron and Ames, 1983). In plate incorporation method the test chemical (0.1 ml), overnight *Salmonella* culture (0.1 ml, 1-2$\times 10^8$ cells) and S9-mix or pH 7.4 phosphate buffer (0.5 ml) were added to a top agar tube, and the content of the tube was vortexed and poured onto 25 ml of minimal glucose agar plate. After the overlay solidified, the plate was inverted and incubated at 37℃ for 48 h. In preincubation method bacteria, test chemical and S9-mix or phosphate buffer were incubated at 37℃ for 20 min without shaking, after then the top agar was added and the same procedures of plate incorporation method were followed. Three plates were used at each dose and experiments were repeated at least 1 week following the initial test for confirming the reproducibility of the results.

Chromosome aberration assay in CHL cells

Cells

Chinese hamster lung fibroblast (CHL) cells were obtained from Dr. T. Sofuni of National Institute of Health Sciences, Tokyo, Japan. The karyotype consists of 25 chromosomes and the doubling time is approximately 15 h (Koyama *et al.*, 1970). The cells were cultured in a CO_2 incubator at 37℃ and approximately 90% humidity with Eagles minimum essential medium (Gibco) supplemented with 10% heat-inactivated fetal bovine serum (Gibco). The subcultures were made every fifth day.

Chemicals and doses

4-Quinolone antibiotics were dissolved in distilled water or 0.02N-NaOH and diluted with the same solvents. Desired final concentrations were prepared by addition of 0.5 ml of the dilutions to the media in 60 mm culture dishes (Falcon). Three doses were prepared by a factor of 2 from the maximum dose at which cell growth was inhibited approximately 50%. Solvent control and positive controls, benzo(a)pyrene (Sigma) with S9-mix or mitomycin-C (Sigma) without metabolic activation, were tested simultaneously.

Chromosome test

CHL cells (1$\times 10^5$ cells/5 ml) were seeded in a 60 mm plastic petri plate. In assay with metabolic activation, when the cultures were 3 days old 2.5 ml of media was discarded and 0.5 ml of chemical solution was added

simultaneously with S9-mix (0.5 ml). The S9-mix contained 30% of S9 fraction (Matsuoka *et al.* 1979). Because of the cytotoxicity of S9 itself, the media was decanted after 6 h incubation and the culture was continued for another 18 h in fresh medium. In assay without metabolic activation the cells were treated with chemicals for 24 h. The cells were harvested after 2 h of treatment with colcemid (0.2 μg/ml, Gibco), and chromosome slides were made according to the conventional air-drying method. When the incidence of cells with chromosome aberrations was over 5%, the experiment was repeated.

Wing spot assay in *Drosophila*

***Drosophila* stocks**

Both strains, *mwh* and flr^3*/TM3, Ser.* were provided by Dr. U. Graf, Institute of Toxicology, Swiss Federal Institute of Technology and University of Zürich, Switzerland. The genetic markers are described by Lindsley and Grell (1968), Garcia-Bellido and Dapena (1974) and Lindsley and Zimm (1985). They had been maintained in well-yeasted culture bottles (28 mm diameter × 100 mm height) containing standard corn meal media (agar 8 g, corn meal 70 g, sugar 100 g, yeast 15 g, ebiose 15 g, propionic acid 4 ml and 1000 ml of distilled water).

Chemicals and doses

Pefloxacin and ciprofloxacin were dissolved in distilled water. The mixture of tween 80 (one part, Sigma) and ethanol (two parts, Merck) were used for suspending ofloxacin, norfloxcain and enoxcin. Their LD_{50} were determined by the number of surviving flies per vial, and three or five doses below LD_{50} were tested.

Wing spot test

Except the only modification of using new strains and standard corn meal medium, all antibiotics were tested and evaluated using the experimental procedures and statistical analysis as described by Graf *et al.* (1984). Virgin females from *mwh* stock were mated with males from flr^3*/TM3, Ser.* stock for the *mwh*-flr^3 cross. The two types of progeny resulting from this mating are (1) *mwh*/flr^3 (non-serrate trans-dihybrid flies), (2) *mwh/TM3, Ser.* (serrate flies heterozygous for both *mwh* and the multiple inversions contained in *TM3*). Eggs from this cross were collected for 8 h in the well-yeasted culture bottles. When the larvae were 72±4 h old the antibiotic solutions were added to the surface of the medium and kept at 25℃ until the adult flies hatched (Marec and Socha, 1987). The flies were collected and stored in 70% ethanol. Their wings were mounted in Faure's solution, and the presence of mosaic spots was inspected under a microscope at 400× magnification.

RESULTS

***Salmonella* mutation assay**

Five 4-quinolones did not show any mutagenicity in

Table I. Mutagenicity of ciprofloxacin in *Salmonella* in preincubation test

Dose (μg/plate)	Solvent	S9-mix	Revertants per plate[a]				
			TA98	TA100	TA1535	TA1537	TA1538
0.00	H_2O	-	19±2.1	121±7.0	7±3.2	10±2.3	11±5.0
0.01			26±1.5	118±3.8	8±2.6	5±1.2	8±3.5
0.03			26±7.8	131±10.6	7±3.5	7±2.1	9±4.0
0.10			25±5.5	116±17.8	6±3.8	6±2.1	8±0.6
0.30			8±2.1	75±12.6	5±1.5	3±2.3	8±4.7
1.00			7±2.1	49±10.6	2±1.0	2±1.5	6±2.9
3.00			0±0.0	0±0.6	3±1.2	0±0.6	2±1.5
Positive control[b]			1381±210.0	>2000	2021±394.6	1037±105.5	1423±86.1
0.00	H_2O	+	26±1.5	129±19.3	9±2.3	9±3.1	13±4.0
0.01			31±6.5	135±16.8	14±5.3	9±2.1	13±9.0
0.03			32±3.8	131±17.9	12±3.5	5±3.1	18±2.1
0.10			26±6.7	151±11.4	7±2.0	7±2.0	16±3.1
0.30			33±7.5	128±17.2	6±2.1	5±1.2	17±2.0
1.00			11±3.6	37±7.9	1±0.6	4±0.6	11±1.5
3.00			0±0.6	0±0.0	0±0.6	2±1.5	6±3.8
Positive control[c]			>2000	>2000	267±25.0	86±4.0	117±12.2

[a]Mean±S.D. for triplicate plates. [b]Strains: positive control (μg/plate, solvent), TA98, TA1538: 2-nitrofluorene (10, DMSO), TA100, TA1535: sodium azide (10, H_2O), TA1537: ICR-191 (1, DMSO). [c]Strains: positive control (μg/plate, solvent), all strains: 2-aminoanthracene (1.5, DMSO).

Table II. Mutagenicity of pefloxacin in *Salmonella* in preincubation test

Dose (μg/plate)	Solvent	S9-mix	Revertants per plate[a]				
			TA98	TA100	TA1535	TA1537	TA1538
0.000	H_2O	-	24±9.5	101±9.2	17±4.0	5±2.1	13±5.5
0.003			20±4.4	91±3.5	13±5.5	8±2.5	11±3.6
0.010			21±4.6	91±9.0	11±4.0	6±2.6	13±2.1
0.030			25±10.1	101±5.5	10±1.2	7±4.0	8±1.0
0.100			29±5.1	108±5.5	10±2.1	11±5.0	10±4.2
0.300			25±2.5	59±12.2	8±2.5	7±1.0	11±4.0
1.000			5±2.0	1±1.2	0±0.6	2±1.0	1±1.5
Positive control[b]			1723±473.6	1617±110.0	1614±357.6	1238±206.4	>2000
0.000	H_2O	+	32±3.2	111±14.1	13±6.4	7±1.5	16±2.5
0.003			27±2.0	125±8.0	11±3.5	8±2.6	15±4.0
0.010			23±6.5	136±7.9	9±4.5	7±2.5	14±1.2
0.030			30±4.7	118±14.0	10±4.2	9±2.1	16±1.0
0.100			38±4.7	121±6.5	8±2.1	9±2.6	13±1.0
0.300			24±8.2	74±9.9	2±0.0	9±3.2	14±2.9
1.000			2±1.7	1±1.7	3±1.4	2±1.7	2±2.5
Positive control[c]			>2000	1788±79.7	225±3.8	386±39.6	153±18.5

[a]Mean±S.D. for triplicate plates. [b]Strains: positive control (μg/plate, solvent), TA98, TA1538: 2-nitrofluorene (10, DMSO), TA100, TA1535: sodium azide (10, H_2O), TA1537: ICR-191 (1, DMSO). [c]Strains: positive control (μg/plate, solvent), all strains: 2-aminoanthracene (1.5, DMSO).

Table III. Mutagenicity of norfloxacin in *Salmonella* in preincubation test

Dose (μg/plate)	Solvent	S9-mix	Revertants per plate[a]				
			TA98	TA100	TA1535	TA1537	TA1538
0.00	H_2O	-	22±7.6	144±21.4	25±6.6	6±1.0	9±4.2
0.01			23±5.3	138±9.5	22±2.0	6±1.5	14±4.9
0.03			25±3.1	162±22.6	16±1.2	8±1.7	10±4.0
0.10			26±11.3	161±24.5	12±3.8	6±4.2	11±6.5
0.30			20±2.5	139±0.6	6±1.2	8±1.0	9±4.4
1.00			6±4.9	62±9.9	4±3.1	5±1.2	4±3.5
3.00			0±0.6	10±11.8	1±0.6	2±2.1	0±0.0
Positive control[b]			1170±222.2	1447±20.0	1737±348.9	939±90.0	1350±213.3
0.00	H_2O	+	42±3.5	197±8.5	20±1.5	5±1.0	16±1.7
0.01			28±2.6	162±6.0	22±4.6	6±2.5	14±2.1
0.03			30±9.5	171±17.1	24±8.1	4±4.0	14±4.0
0.10			36±0.0	210±25.5	18±5.5	7±1.0	10±2.1
0.30			21±1.2	196±2.6	13±4.6	5±1.5	19±12.4
1.00			11±4.0	73±34.8	4±2.1	5±1.0	17±2.0
3.00			0±0.0	5±6.1	1±1.0	0±0.6	0±0.0
Positive control[c]			1850±321.6	>2000	267±12.1	92±35.2	158±12.5

[a]Mean±S.D. for triplicate plates. [b]Strains: positive control (μg/plate, solvent), TA98, TA1538: 2-nitrofluorene (10, DMSO), TA100, TA1535: sodium azide (10, H_2O), TA1537: ICR-191 (1, DMSO). [c]Strains: positive control (μg/plate, solvent), all strains: 2-aminoanthracene (1.5, DMSO).

Salmonella typhimurium TA98, TA100, TA1535, TA1537 and TA1538 in preincubation test (Table I~Table V). In *Salmonella typhimurium* TA102, all 4-quinolones induced reproducible and dose-related increase of *his*[+] revertants by two to three fold in plate incorporation test (Fig. 2) and three to four fold over solvent control in preincubation method (Fig. 3) both with and without metabolic activation. They exhibited their strongest mutagenic responses at the dose just below the growth inhibiting concentration, which was same both in preincubation method and plate incorporation method except ofloxacin which caused growth inhibition at lower dose

Table IV. Mutagenicity of ofloxacin in *Salmonella* in preincubation test

Dose (μg/plate)	Solvent	S9-mix	Revertants per plate[a]				
			TA98	TA100	TA1535	TA1537	TA1538
0.000	H_2O	-	18±7.4	131±10.7	25±5.9	10±1.5	8±4.0
0.003			25±5.9	111±10.5	20±3.0	6±1.2	5±4.0
0.010			23±1.5	133±7.8	12±2.5	5±0.6	10±3.1
0.030			24±0.6	116±6.9	7±1.5	7±1.5	10±4.6
0.100			13±4.0	52±22.1	3±1.5	2±2.5	6±2.1
0.300			7±3.2	12±2.5	1±1.5	3±1.0	0±0.0
1.000			0±0.0	0±0.0	1±0.6	0±0.6	0±0.0
Positive control[b]			1096±27.6	1833±224.1	1656±176.8	1103±103.0	1288±248.7
0.000	H_2O	+	29±6.6	114±7.5	18±2.6	11±4.0	15±1.7
0.003			33±6.2	125±15.1	20±4.7	10±2.1	9±2.5
0.010			42±5.5	133±25.5	13±1.7	11±3.0	12±2.1
0.030			40±11.5	149±7.2	16±1.7	8±5.1	16±1.7
0.100			28±7.6	59±16.5	3±4.9	5±2.1	11±1.2
0.300			5±3.1	22±11.5	2±0.6	2±1.6	2±0.0
1.000			0±0.0	23±2.5	2±1.7	0±0.6	0±0.0
Positive control[c]			2650±450.5	>2000	268±19.1	176±25.0	153±7.8

[a]Mean±S.D. for triplicate plates. [b]Strains: positive control (μg/plate, solvent), TA98, TA1538: 2-nitrofluorene(10, DMSO), TA100, TA1535: sodium azide (10, H_2O), TA1537: ICR-191 (1, DMSO). [c]Strains: positive control (μg/plate, solvent), all strains: 2-aminoanthracene(1.5, DMSO).

Table V. Mutagenecity of enoxacin in *Salmonella* in preincubation test

Dose (μg/plate)	Solvent	S9-mix	Revertants per plate[a]				
			TA98	TA100	TA1535	TA1537	TA1538
0.00	H_2O	-	23±3.0	109±5.2	7±2.5	10±1.7	9±2.5
0.01			21±2.6	113±13.8	7±2.0	8±1.0	16±6.1
0.03			26±4.7	107±18.2	11±4.0	15±5.3	10±1.7
0.10			24±2.5	130±10.4	8±5.2	7±4.5	12±4.6
0.30			28±4.2	146±1.5	8±4.9	6±2.1	15±4.6
1.00			25±4.5	150±18.1	5±1.0	5±0.7	12±5.6
3.00			9±15.6	46±9.9	3±1.5	9±4.0	6±4.4
Positive control[b]			1784±376.2	1491±102.5	1655±264.8	995±3.1	1515±86.9
0.00	H_2O	+	34±8.3	102±3.1	9±4.5	14±2.5	19±4.0
0.01			36±9.5	105±13.1	14±1.5	10±0.0	19±6.7
0.03			29±3.5	122±11.1	11±7.1	7±3.5	15±5.3
0.10			29±0.6	133±17.5	8±2.1	9±0.6	20±3.2
0.30			36±3.5	129±1.5	8±1.2	9±6.1	24±4.6
1.00			52±14.1	161±19.7	4±2.9	11±2.3	18±2.6
3.00			0±0.0	8±13.9	1±1.7	7±5.3	5±3.5
Positive control[c]			>2000	1893±88.0	270±17.6	430±3.6	248±18.2

[a]Mean±S.D. for triplicate plates. [b]Strains: positive control (μg/plate, solvent), TA98, TA1538: 2-nitrofluorene (10, DMSO), TA100, TA1535: sodium azide (10, H_2O), TA1537: ICR-191 (1, DMSO). [c]Strains: positive control (μg/plate, solvent), all strains: 2-aminoanthracene (1.5, DMSO).

in preincubation test.

Chromosome aberration assay

Following criteria were used for evaluation of chromosome aberration: negative (-) less than 5%; inconclusive (+/-), 5% to less than 10%; positive (+) more than 10% (Ishidate, 1983).

Table VI and Table VII summarize the results of chromosome aberration assay. 4-Quinolone antibiotics induced chromatid type of aberrations predominantly but rare chromosome breaks and gaps. Breaks narrower than the width of a chromatid were grouped in gaps. Ciprofloxacin induced structural aberrations in 28% of the

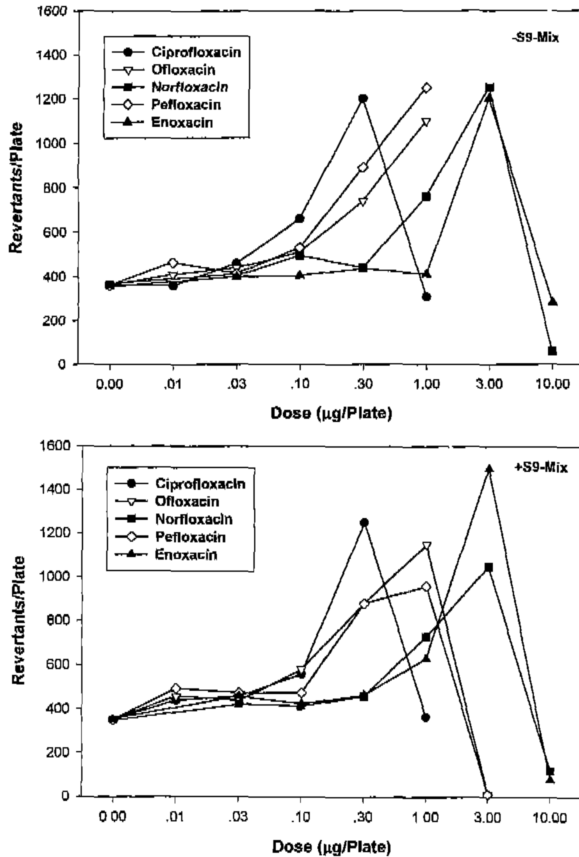

Fig. 2. Mutagenicity of 4-quinolone antibiotics in *Salmonella typhimurium* TA102 without (a) and with (b) metabolic activation in plate incorporation test.

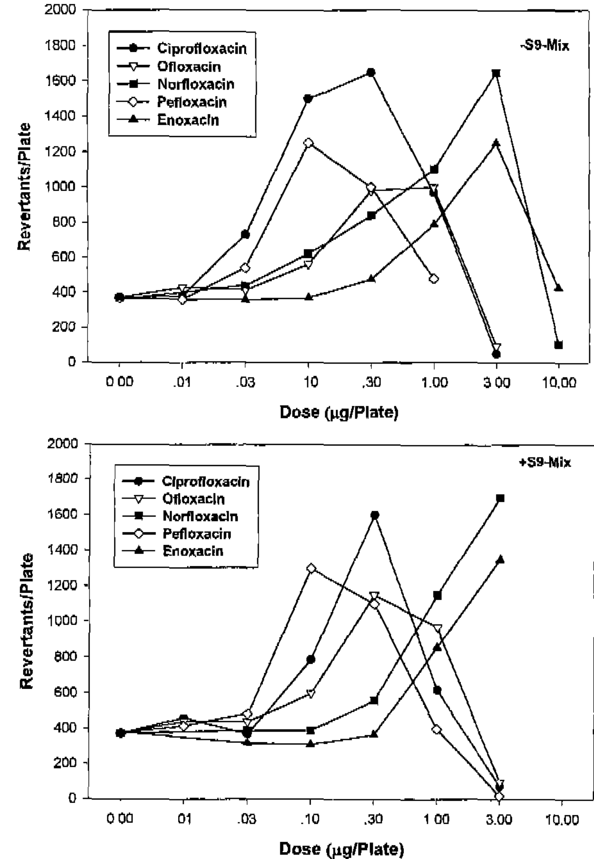

Fig. 3. Mutagenicity of 4-quinolone antibiotics in *Salmonella typhimurium* TA102 without (a) and with (b) metabolic activation in preincubation test.

Table VI. Frequencies of structural chromosome aberration in Chinese hamster lung cells induced by 4-quinolone antibiotics without metabolic activation

Chemical	Solvent	S9-mix	Dose (μg/ml)	Treatment time (h)	No. of cells scored	Frequencies of aberration						Total frequencies of aberrant cells (%)
						Ctg	Ctb	Cte	Csg	Csb	Cse	
Control (H_2O)												
Ciprofloxacin	H_2O	-	500	24	100	5	15	19	0	2	0	28
			250		100	0	8	6	1	0	0	10
			125		100	0	1	0	0	0	0	1
Pefloxacin	H_2O	-	500	24	20	0	1	0	0	0	0	5
			250		100	2	0	0	1	0	0	4
			125		100	1	2	0	1	0	0	3
Norfloxacin	0.02N-NaOH	-	500	24	100	2	0	0	0	1	0	3
			250		100	1	0	0	2	0	0	3
			125		100	1	0	0	0	0	0	1
Ofloxacin	0.02N-NaOH	-	500	24	100	0	2	0	2	0	0	4
			250		100	4	0	1	1	0	0	4
			125		100	0	0	0	0	0	0	0
Enoxacin	0.02N-NaOH	-	500	24	100	0	1	0	0	0	0	1
			250		100	1	0	0	1	0	0	2
			125		100	0	0	1	0	0	0	1
Mitomycin C	Saline	-	0.1	24	100	2	16	10	1	2	1	28

Ctg: chromatid gaps, Ctb: chromatid breaks, Cte: chromatid exchanges, Csg: chromosome gaps, Csb: chromosome breaks, Cse: chromosome exchanges including dicentric and ring chromosome.

Table VII. Frequencies of structural chromosome aberration in Chinese hamster lung cells induced by 4-quinolone antibiotics with metabolic activation

Chemical	Solvent	S9-mix	Dose (μg/ml)	Treatment time (h)	No. of cells	Frequencies of aberration						Total frequencies of aberrant cells (%)
						Ctg	Ctb	Cte	Csg	Csb	Cse	
Control (H_2O)		+		24	100	0	0	0	0	0	0	0
Ciprofloxacin	H_2O	+	500	6	100	6	1	0	0	0	0	6
			250		100	2	1	0	0	0	0	3
			125		100	2	0	0	0	0	0	2
Pefloxacin	H_2O	+	500	6	100	3	3	0	0	0	0	6
			250		100	2	1	0	1	0	0	4
			125		100	1	0	0	0	0	0	1
Norfloxacin	0.02N-NaOH	+	500	6	100	0	0	1	0	0	0	1
			250		100	0	0	1	0	0	0	1
			125		100	2	0	0	0	0	0	2
Ofloxacin	0.02N-NaOH	+	500	6	100	1	0	0	0	0	0	1
			250		100	2	0	0	0	0	0	2
			125		100	1	0	0	0	0	0	1
Enoxacin	0.02N-NaOH	+	500	6	100	1	0	0	0	0	0	1
			250		100	0	0	0	1	0	0	1
			125		100	0	0	0	0	0	0	0
Benzo(a)pyrene	DMSO	+	200	6	100	22	3	5	5	1	1	38

Ctg: chromatid gaps, Ctb: chromatid breaks, Cte: chromatid exchanges, Csg: chromosome gaps, Csb: chromosome breaks, Cse: chromosome exchanges including dicentric and ring chromosome.

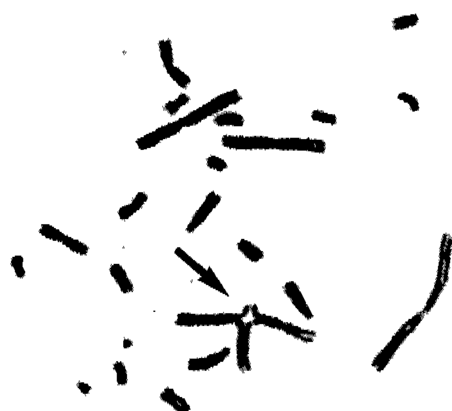

Fig. 4. A chromatid exchange (arrow) induced by 500 μg/ml of ciprofloxacin without metabolic activation.

cells without metabolic activation (Fig. 4) and showed inconclusive result of 6% in the presence of S9-mix. Pefloxacin had an inconclusive result both with and without metabolic activation, while the others did not show any increase of structural aberrations. The high dose (500 μg/ml) of pefloxacin without metabolic activation was so toxic that only twenty cells could be scored.

Wing spot assay

Table VIII shows the results of the wing spot assay using the trans-dihybrid flies. The spots were grouped into 3 types: small single spots of 1 or 2 cells in size (*mwh* or *flr*); large single spots of 3 and more cells (*mwh* or *flr*); twin spots with *mwh* and *flr* area. Ofloxacin increased the frequency of small single spots dose-dependently but there was no dose-dependent increase of single or twin spots in the others, although pefloxacin caused large single spots at a low concentration (14 mg/ml) and norfloxacin induced twin spots at 25 mg/ml. Fig. 5 shows the twin spots induced by 50 mg/ml ofloxacin.

DISCUSSION

In *Salmonella* mutagenicity assays, nalidixic acid and other 4-quinolones have negative results in *Salmonella typhimurium* TA 98, TA100, TA1535, TA1537 or TA 1538 (McCoy *et al.*, 1980; Irikura and Hosomi, 1981; Shimada *et al.*, 1984; Schlüter, 1986; Hosomi *et al.*, 1988). We, too, confirmed the absence of the mutagenicity of five 4-quinolone antibiotics in *Salmonella typhimurium* TA98, TA100, TA1535, TA1537 and TA 1538 in this study.

In TA102 strain, however, the mutagenic response of

Table VIII. Wing spot data from *mwh+/+flr*[3] *Drosophila* larvae exposed to 4-quinolone antibiotics

Chemical	Solvent	Dose (mg/ml)	% Frequency per wing(number) of			Total number of wings with spots	Number of wings scored
			Single spots		Twin spots		
			Small	Large			
Control							
H_2O			5.6 (13)	2.6 (6)	0.9 (2)	21	232
Solvent mix[a]			1.5 (2)	0.8 (1)	0.0 (0)	3	130
Ciprofloxacin	H_2O	35	3.3 (3)	0.0 (0)	0.0 (0)	3	90
		17	10.3 (6)	1.7 (1)	3.4 (2)	9	58
		9	0.0 (0)	0.0 (0)	0.0 (0)	0	67
Pefloxacin	H_2O	106	0.0 (0)	0.0 (0)	0.0 (0)	0	26
		53	0.0 (0)	0.0 (0)	0.0 (0)	0	15
		27	0.0 (0)	2.3 (1)	0.0 (0)	1	43
		14	1.9 (1)	9.3 (5)*	0.0 (0)	6	54
		7	0.0 (0)	0.0 (0)	0.0 (0)	0	33
Norfloxacin	Solvent mix	50	3.7 (4)	1.8 (2)	1.8 (2)	8	109
		25	3.8 (2)	0.0 (0)	5.8 (3)*	5	52
		13	3.2 (3)	3.2 (3)	0.0 (0)	6	95
Ofloxacin	Solvent mix	50	10.2 (10)*	2.0 (2)	2.0 (2)	14	98
		25	8.9 (9)*	3.0 (3)	0.0 (0)	12	101
		13	4.0 (4)	2.0 (2)	2.0 (2)	8	100
Enoxacin	Solvent mix	50	0.0 (0)	0.0 (0)	0.0 (0)	0	27
		25	1.8 (1)	0.0 (0)	0.0 (0)	1	57
		13	3.0 (3)	0.0 (0)	2.0 (0)	3	101

[a]Tween 80 : EtOH=1 : 2 mixture. *Level of significance: $p<0.05$ by χ^2 test.

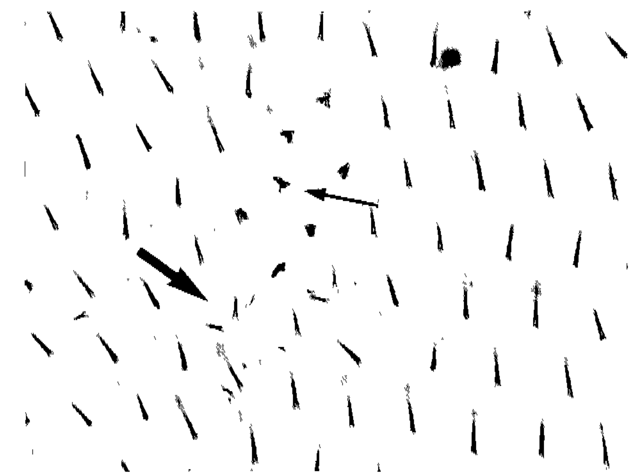

Fig. 5. Twin spots (thin arrow: *flr*, thick arrow: *mwh*) induced by 50 mg/ml ofloxacin (×800).

4-quinolones have been reported (Power and Phillips, 1993; Mamber *et al.*, 1993; Albertini *et al.*, 1995), and it is suggested that a functional excision repair system is essential for quinolone-induced bacterial mutagenesis, and the mechanism is SOS-processed DNA damage (Clerch *et al.*, 1992; Gocke, 1991; Power and Philips, 1993). However, they did not specify the method, whether it was plate incorporation method or preincubation method. In this study we used both methods to compare the mutagenic response of 4-quinolones in both. Preincubation method was known to have equal or greater sensitivity than the plate incorporation method (Matsushima *et al.*, 1980). As it was expected the mutagenic response of preincubation test was higher than that of plate incorporation method in our test. The reason would be due to preincubation method allows interaction of test compound, S9 and bacteria at higher concentration during incubation than the plate incorporation method (Prival, 1979).

The capacity of 4-quinolones to affect the eukaryotic cellular DNA has been described by several reports. The influence on mammalian DNA enzyme has been detected by Hussy *et al.*, (1986). The induction of unscheduled DNA synthesis by 4-quinolones in rat cells *in vitro* has been reported by McQueen and Williams (1987). According to Bredberg *et al.*, (1989), ciprofloxacin induces a significant amount of unscheduled DNA synthesis, and the induction of DNA strand breakage in lymphoblastoid cells has been found to be in order of

ciprofloxacin (10 μg/ml and upwards), ofloxacin (80 μg/ml) and norfloxacin (160 μg/ml). Ciprofloxacin and other 4-quinolones strongly enhance the incorporation [^{3}H] thymidine into mitogen stimulated peripheral blood lymphocytes (Forsgren *et al.*, 1987; Bredberg *et al.*, 1989). Therefore, in this study, the induction of chromosomal aberrations by only ciprofloxacin seems to be due to its strongest effects on the cellular DNA, and its weak response in metabolic activation system could be thought because of short treatment (6 h). The results on chromosome aberration induced by ciprofloxacin is comparable to the result using V79 CHL cells in direct system (Curry *et al.*, 1996). We carried out metabolic activation system as well as direct system, and we found dose-dependent incerease of chromosome aberrations. Hosomi *et al.* (1988) has reported that norfloxacin has no detectable genotoxicity in battery of *in vivo* tests. Other have found no evidence of chromosome aberrations *in vitro* or *in vivo* by norfloxacin (Irikura *et al.*, 1981; Irikura and Hosomi, 1981) and ofloxacin (Shimada, 1984). Our study showed no chromosomal abberation induction of norfloxacin, ofloxacin and enoxacin, either.

Drosophila is an eukaryotic *in vivo* assay system with a versatile endogenous metabolic activation system (Vogel, 1975; Vogel *et al.*, 1976). Mutagenicity of nalidixic acid reported by Filippova and Efremova (1974) using sex-linked recessive lethal test has been the only study using *Drosophila* system onto mutagenicity of 4-quinolones. The wing spot assay, measuring somatic mutation and mitotic recombination in *Drosophila* (Graf *et al.*, 1984; 1989), has higher sensitivity (0.75-0.78) and accuracy (0.83-0.86) compared with the sex-linked recessive lethal assay (sensitivity 0.33-0.79; accuracy 0.50-0.73, Vogel, 1987). In our study, ofloxacin induced small single spots dose-dependently. Single spots can result from the following events: first, *mwh* single spots from recombination between *flr*^{3} and *mwh*; second, single spots of either *mwh* or *flr*^{3} phenotype from nonrecombinogenic events such as mutation or deletion of the respective wild-type allele (Graf *et al.*, 1984).

Although the 4-quinolone antibiotics are not cytogenic in human with clinical dosage even after long term treatment (Mitelman *et al.*, 1988), our results add the information that a possible genotoxic potential can not be excluded. The mutagenicity is thought to be related to their therapeutic activity and the differences in potential seem to be due to their structures.

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