J. Biochem. Mol. Biol. Vol. 28, No. 5 pp. 464~470 September 30, 1995

Cleavable Complex Formation as a Major Cellular Process in the Antibacterial Action of Quinolones

Ji-Soo Park, Sanghee Park[1], Yeonhee Lee[1], Jae-Yang Kong[2], Wan Joo Kim[2#], Hyeon-Sook Koo*

Department of Biochemistry, College of Science, Yonsei University, Seoul 120-749
[1]Department of Biology, Seoul Woman's University, Seoul 139-744
[2]Research Institute of Chemical Technology, Taejon 305-606, Korea
(Received June 22, 1995)

Abstract: Quinolone antibiotics are DNA gyrase inhibitors, but their bactericidal action seems to involve more than the inhibition of DNA gyrase activity. Hence, the potentially crucial factors among possible mechanisms of quinolone action; cleavable complex formation, inhibition of DNA synthesis, and induction of SOS response were investigated. These parameters were measured in an *Escherichia coli* strain exposed to quinolones in the logarithmic growth phase, and correlated with the bactericidal activity of quinolones. Cleavable complex formation proved to be the factor most related to bactericidal action. Inhibition of DNA synthesis was substantially correlated with bactericidal activity, but induction of SOS response was least correlated with bactericidal activity. Therefore, it was concluded that quinolones exert bactericidal action primarily through cleavable complex formation, and subsequent unknown cellular processes together with inhibition of DNA synthesis contribute to the bactericidal activity of quinolones.
Key words: antibacterial action, DNA gyrase, quinolone.

DNA gyrase (EC 5.99.1.3) in bacteria affects various cellular processes involving DNA, such as replication, transcription, and recombination by controlling DNA superhelicity (reviewed by Gellert, 1981; Wang, 1985; Wang, 1987). DNA gyrase generates negative DNA supercoils and removes positive DNA supercoils with concomitant ATP hydrolysis. The enzyme has a composition of A_2B_2, where A is a catalytic subunit and B is a ATP-hydrolyzing subunit. Quinolones inhibit the enzyme by trapping the intermediate reaction product, the so called cleavable complex (Drlica, 1988; Hooper, 1993). In the cleavable complex a tyrosine residue of DNA gyrase is covalently bonded to the broken DNA ends, concealing the DNA breaks (Horowitz and Wang, 1987). Quinolones are thought to bind to a presumptive single-stranded DNA region generated by DNA gyrase (Shen and Pernet, 1985; Shen *et al.*, 1989).

Quinolones, the inhibitors of the DNA gyrase A subunit have a rapid bactericidal activity, a low occurrence of resistant bacteria, and a broad spectrum against gram-positive and gram-negative bacteria (Neu, 1988). However, although they are DNA gyrase inhibitors, quinolones are thought to exert bactericidal activity through mechanisms somewhat different from DNA gyrase inhibition. One of the reasons is that the minimum inhibitory concentrations are 1/10 to 1/100 of the 50 % inhibitory concentration of DNA supercoiling activity (Hooper & Wolfson, 1988; Zweerink & Edison, 1986). Upon uptake of quinolones, negative DNA superhelicity of bacterial chromosomes decreases due to inactivation of DNA gyrase. The decreased negative superhelicity lowers the initiation efficiency in replication and generally in transcription (Gellert, 1981; Wang, 1985; Wang, 1987). The cleavable complex may also impede the movement of DNA and RNA polymerases in the elongation steps of replication and transcription, respectively. For example, in a cell-free SV40 replication system, aberrant replication products are formed through the collision of a replication fork with the cleavable complex of eukaryotic DNA topoisomerase I (Hsiang *et al.*, 1989; Tsao *et al.*, 1993). Inhibition of bacterial DNA synthesis by quinolones is probably exerted through blockage of replication forks and also through inefficient replication initiation.

Quinolones induce SOS response due to DNA damage in cleavable complex formation via the normal pathway of RecA activation (Huisman & D'Ari, 1981; Phillips *et al.*, 1987; Piddock *et al.*, 1990). In SOS

*To whom correspondence should be addressed.
Tel: 82-2-361-2701, Fax: 82-2-362-9897.
Present address: Hanmi Pharm. Co. Ltd., Seoul 138-050, Korea.

response, at least 17 genes participating in DNA repair are induced (Walker, 1984). Among them, the *sfiA* gene, the product of which is a cell division inhibitor, is induced to stop cell division during DNA repair (Huisman & D'Ari, 1981; Huisman & D'Ari, 1983; Huisman *et al.*, 1984). Although the SfiA protein has a short half-life time, the long-period induction of the *sfiA* gene is lethal to bacteria (Maguin *et al.*, 1986). Therefore, it has been proposed that induction of SOS response is thought to be, in part, a mechanism of quinolone action. (Phillips *et al.*, 1987; Piddock *et al.*, 1990).

Among the various cellular responses against quinolones, the one which plays a crucial role in the cell killing pathway is not certain. Rapid inhibition of DNA synthesis has been generally accepted as the primary cause of cell death. However, the fact that the bactericidal activity of quinolones is repressed by rifampin and chloramphenicol, unlike inhibition of DNA synthesis, suggests that other processes besides inhibition of DNA synthesis may be involved in the cell killing pathway (Deitz *et al.*, 1966). So, the mechanism which is most responsible for the bactericidal action of quinolones was investigated. The bactericidal activity of each quinolone agent was compared with its ability to form a cleavable complex, to inhibit DNA replication, and to induce SOS response. Correlations were made between bactericidal activity and each of the above factors. According to the results, the bactericidal action of quinolones is most related to cleavable complex formation.

Materials and Methods

Materials

E. coli PB40 (MC4100, *zei-298*::Tn10, λ*cIind sfiA*::*lacZ*) was obtained from Dr. Martine Couturier of the Université Libre de Bruxelles, Belgium. Ciprofloxacin and ofloxacin were from Bayer Pharmaceutical Co. and Jae-il Phar. Co. (Seoul, Korea), respectively. Other quinolones used in this work were purchased from the Sigma Chemical Co. Lysozyme, sodium dodecyl sulfate, proteinase K, dithiothreitol, deoxynucleoside triphosphates, thymidine, and o-nitrophenyl β-D-galactopyranoside were purchased from the Sigma Chemical Co. Klenow fragment was supplied by Promega and GF/C filters (AA disc, 6.0 mm) were purchased from Whatman. [α-^{32}P]dCTP and [^{3}H]thymidine were supplied by Amersham. Bacto agar, bacto tryptone, and yeast extract were Difco (Detroit, USA) products.

E. coli culture

E. coli PB40 cells were grown in LB medium and treated with quinolones in the logarithmic growth phase ($O.D._{600\ nm}=0.4$), except cells used for measurement of DNA synthesis ($O.D._{600\ nm}=1.0$).

Bactericidal activity

The bactericidal activity of quinolones was measured by cell killing kinetics. *E. coli* PB40 cells were grown to the logarithmic phase in LB medium at 37℃, then each of the six quinolones was added in appropriate concentrations, followed by a further incubation for 30 min. Cultures were rapidly cooled on ice, then centrifuged. Harvested cells were washed with phosphate-buffered saline (PBS) once, then diluted 10,000 times with PBS solution. A cell suspension of 100 μl was spread on an agar plate (dia. 8.7 cm), then incubated at 37℃ overnight. The number of colonies was counted and percent nonviability was calculated with respect to the number of colonies lacking drug treatment.

Cleavable complex formation

E. coli PB40 cells were grown to the logarithmic phase, then divided into aliquots of 30 ml in 37℃ prewarmed culture tubes containing appropriate concentrations of a quinolone. The aliquots were further incubated for 5 min at 37℃, then rapidly cooled on ice. Bacteria were harvested and the cell pellets were resuspended in 200 μl of a buffer (25 mM Tris-Cl, pH 8.0, 50 mM glucose, 10 mM EDTA). To the cell suspensions, 50 μl of 0.25 M EDTA and 50 μl of 10 mg/ml lysozyme were added, and incubation continued for 10 min at 4℃. To the lysate, 50 μl of 10% SDS and 20 μl of 2 mg/ml proteinase K were added, and the reaction was continued at 37℃ for 7 h. After two phenol extractions, RNase A digestion (100 μg/ml) was performed at 37℃ for 20 min, followed by phenol extraction and ethanol precipitation. The DNA pellets were resuspended in 20 μl of TE (pH 8.0) buffer. The amount of isolated chromosomal DNA was measured by mixing the DNA with Hoechst 33258 dye, then measuring the fluorescence (Kontron SFM 25 fluorometer).

Isolated chromosomal DNA (3.0 μg) was incubated at 37℃ for 30 min in a buffer (20 μl) containing 50 mM Tris-Cl (pH 7.6), 10 mM $MgCl_2$, 1 mM dithiothreitol, 25 μM each of dATP, dTTP, and dGTP, 10 μCi of [α-^{32}P]dCTP (3,000 Ci/mmol), and 5 U of Klenow fragment. The reaction was stopped by addition of 1 ml of 5% ice-cold trichloroacetic acid and the reaction mixture was kept on ice for 1 h. Acid insoluble material was collected by centrifugation at 14,000 rpm in a microcentrifuge for 1 h, then the material was resuspended in 50 μl of TE buffer (pH 8.0). Precipitation in 5% trichloroacetic acid was repeated twice and washed DNA pellets were resuspended in 50 μl of TE buffer. After addition of 10 ml of a cocktail solution to each

sample, radioactivity was measured using a liquid scintillation counter (Packard, TRI-CARB 1900-TR).

Inhibition of DNA synthesis

E. coli PB40 cultures ($O.D._{600\ nm}=1.0$) were pretreated with quinolones for 5 min. [^{3}H]thymidine (10 μCi, 70 Ci/mmol) was added to 1 ml cultures and incubation was continued for 10 min before stopping the reaction by adding 1 ml of an ice-cold 10% trichloroacetic acid solution. The reaction mixture was kept on ice for 30 min, then run through a GF/C filter fixed in a vacuum manifold (Bio-Rad). GF/C filters were presoaked in a 0.05% nonradioactive thymidine solution to block nonspecific adsorption of [α-^{3}H]thymidine. Filters were serially washed with 5 ml each of cooled 5% trichloroacetic acid, 0.1 N HCl, and 95% ethanol. Radioactivity on the filters was counted using a liquid scintillation counter (Packard, TRI-CARB 4530).

Induction of SOS response

Induction of SOS response in *E. coli* PB40 was measured using the method of Huisman & D'Ari (1981). *E. coli* PB40 cells were grown to the logarithic phase and divided into 5 ml aliquots in prewarmed culture tubes containing appropriate concentrations of quinolones. The aliquots were further incubated for 30 min, then rapidly cooled on ice. After harvesting the cultures by centrifugation at 4℃, β-galactosidase activity was measured by the method of Miller (1972) with chloroform and SDS as lysing agents.

Results

E. coli PB40 (MC4100, *zei-298*::Tn10, λ*clind sfiA*::*lacZ*) cells were used to examine various cellular processes upon exposure to quinolones. The strain has a *sfiA* promoter fused with the coding region of the *lacZ* gene so that induction of SOS response can be easily detected by measuring expressed β-galactosidase activity (Bernard and Couturier, 1992). The bactericidal potency of quinolones was measured by counting the surviving number of *E. coli* PB40 cells after 30 min growth in the presence of quinolones. The percentages of nonviable cells were plotted against the concentrations of quinolones added to the *E. coli* cultures (Fig. 1). Ciprofloxacin was the most potent drug, killing almost all cells at concentrations higher than 0.1 μg/ml. Nalidixic acid was the least efficient drug, killing only 17% of the cells at 10 μg/ml. Ofloxacin, norfloxacin, enoxacin, and oxolinic acid all showed cell killing efficiencies between the two extremes, in the listed order. Bactericidal efficiency was represented by the percentage of nonviable cells instead of by minimum inhibitory concentrations because the percentage of nonviable cells was more quantitative and appropriate for comparison with other parameters measured in this study.

Quinolones inhibit DNA gyrase by reversibly forming a ternary complex with DNA and gyrase. In the ternary complex, double-stranded DNA breaks are produced by gyrase, but resealing is interfered by quinolones (reviewed by Drlica, 1988; Hooper, 1993). In order to compare the ability of quinolones to induce gyrase-mediated DNA cleavages, *E. coli* PB40 cells treated with quinolones were lysed by the addition of lysozyme, then sodium dodecyl sulfate. Sodium dodecyl sulfate denatures DNA gyrase in a cleavable complex producing irreversible double stranded DNA breaks. Proteinase K cleaves the denatured DNA gyrase off the DNA ends staggered by 4 base pairs (Lockshon and Morris, 1985). To quantify the extent of gyrase-mediated and quinolone-induced DNA cleavage, the generated 3′ recessive DNA ends were filled with [α-^{32}P]dCTP using Klenow fragment. Radioactivity incorporated in a fixed amount of chromosomal DNA has a parallel relationship with the extent of gyrase-mediated DNA-breaks. The relative extent of DNA cleavage is plotted for quinolones at various concentrations in Fig. 2. The ranking order of quinolones in a capacity for production of chromosomal DNA cleavage is exactly the same as for the bactericidal efficiency illustrated in Fig. 1.

The extent of chromosomal DNA synthesis after preincubating *E. coli* PB40 cells with quinolones was measured by counting the radioactivity of [α-^{3}H]thymidine incorporated into chromosomal DNA. The percent inhibition of DNA synthesis is plotted for different concentrations of quinolones in Fig. 3. At 0.1 μg/ml, ciprofloxacin inhibited DNA synthesis 57%, while ofloxacin inhibited synthesis only 8.7%. On the contrary, the other four quinolones increased DNA synthesis slightly, which is thought to be due to induction of the DNA repair system. When the preincubation time with quinolones at 0.1 μg/ml was lengthened from 5 min to 30 min, increases in DNA synthesis were observed for all quinolones tested except nalidixic acid (data not shown). This suggests further activation of the cellular DNA repair system due to longer drug treatment. Ciprofloxacin, ofloxacin, norfloxacin, and enoxacin nearly eliminated DNA synthesis at 1 μg/ml. Oxolinic acid and nalidixic acid at 1 μg/ml slightly inhibited DNA synthesis to a similar extent, while at 10 μg/ml oxolinic acid was more inhibitory than nalidixic acid.

As an indicator of induction of SOS response by quinolones, changes in expression from the *sfiA* promoter were measured as shown in Fig. 4. Transcription of the *sfiA* gene is repressed by the LexA protein, and the expressed protein prevents cell septation (Huisman

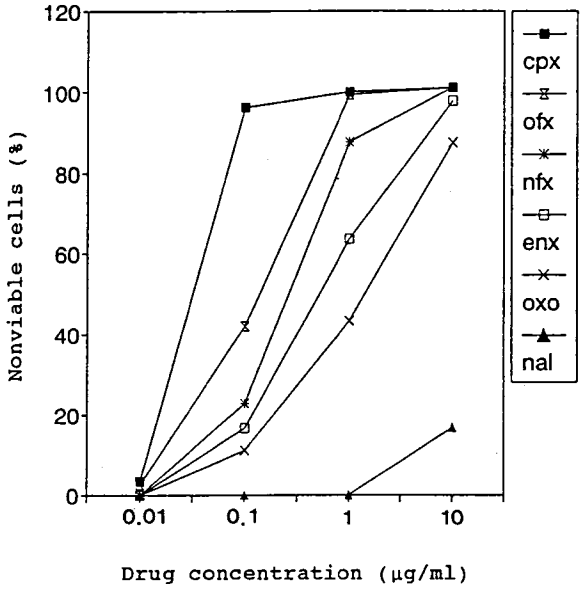

Fig. 1. Bactericidal activity of quinolones at various concentrations. *E. coli* PB40 cells in the logarithmic growth phase were treated with quinolones for 30 min, then viable counts were estimated by plating the cells on nutrient agar. The average standard deviation of nonviability was 4.0%.

and D'Ari, 1983; Huisman *et al.*, 1984). In *E. coli* PB 40 the *sfiA* promoter exists in the chromosome fused with the coding region of the β-galactosidase gene. Relative extents of *sfiA* induction were measured by the activity of β-galactosidase in permeabilized cells, after incubating the cells with quinolones for 30 min. Ciprofloxacin significantly induced the *sfiA* gene at 0.01 μg/ml, however, it did not cause much increase in induction at higher concentrations. Ciprofloxacin had a lower *sfiA* gene induction at 10 μg/ml than at 1 μg/ml. A similar contradicting decline in bactericidal efficiency was also observed above the highest bactericidal concentrations of quinolones (Walters *et al.*, 1989; Piddock *et al.*, 1990). This phenomenon at high quinolone concentrations is thought to originate from an inhibition of transcription by quinolones (Phillips *et al.*, 1987). Ofloxacin was the second most efficient quinolone having an extent of *sfiA* gene induction similar to ciprofloxacin at 0.1 μg/ml. Enoxacin and norfloxacin produced significant increases in induction between 0.1 and 1 μg/ml. Oxolinic acid did not significantly induce the *sfiA* gene at concentrations up to 1 μg/ml, but it showed a great increase in induction at 10 μg/ml, having a greater effect than norfloxacin. Nalidixic acid was the least efficient drug in induction as in bactericidal activity and cleavable complex formation. Quinolones caused gradual increases in induction with elapsed times up to 1 h at 1 μg/ml (data not shown). However, at 10 μg/ml of oxolinic acid, *sfiA* gene induction began to diminish after 90 min (data not shown).

Discussion

Quinolones kill bacteria primarily by acting as inhibitors of DNA gyrase, which is an essential enzyme to maintain the negative superhelicity of chromosomal DNA. Because negative DNA supercoiling promotes initiation of DNA replication, recombination, and usually initiation of RNA transcription, these cellular processes are under the indirect control of DNA gyrase (Gellert, 1981; Wang, 1985; Wang, 1987). Besides these indirect roles, DNA gyrase actively participates in the elongation step of transcription by removing excessive positive DNA supercoils generated in front of RNA polymerase (Wu *et al.*, 1988). DNA gyrase is also thought to be crucial in the segregation of replicated daughter DNA molecules in bacteria, based on the role of DNA topoisomerase II in yeasts (Dinardo *et al.*, 1984; Uemura *et al.*, 1987) and also in an *in vitro* SV40 DNA replication system (Yang *et al.*, 1987). Although DNA gyrase is a major cellular target of quinolones it remains to be determined how quinolones cause cell death. In order to find a cellular process which plays a critical role in the cell killing pathway, the efficiencies of quinolones in producing cellular responses were examined, and these efficiencies were compared with bactericidal efficiencies.

As shown in Figs. 1 to 4, various cellular reactions occur in response to quinolone uptake. In order to determine the most important cellular reaction contributing to cell death, the bactericidal activity illustrated in Fig. 1 was correlated with chromosomal DNA cleavage, inhibition of DNA synthesis, and induction of the *sfiA* gene shown in Figs. 2 to 4. Correlations, plotted in Fig. 5, were made between data points corresponding to identical concentrations of the same drug. Among the three parameters correlated with bactericidal activity (represented by percent nonviable cells) chromosomal DNA cleavage had the highest correlation coefficient of 0.9498. In Fig. 5A which shows the correlation between percent nonviable cells and chromosomal DNA cleavage, five data points beyond DNA cleavage at 3,000 cpm were excluded in calculating the correlation coefficient. Even though these data points deviated from the linear correlative relationship, they indicated a perfect correlation in a biological sense, in that all cells lost viability above that level of chromosomal DNA cleavage. The correlation of percent nonviability with inhibition of DNA synthesis shown in Fig. 5B illustrates a polarization of data points which is mainly due to a lack of data points at medium levels of DNA synthesis inhibition. However, inhibition of

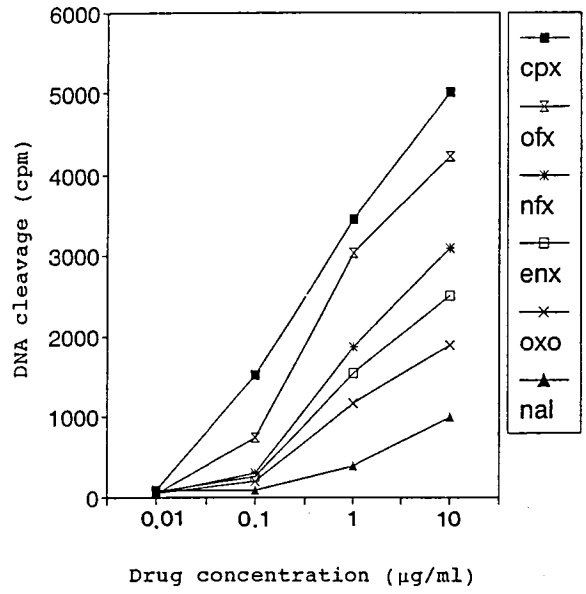

Fig. 2. Cleavable complex formation in *E. coli* PB40 at various concentrations of quinolones. After growing *E. coli* PB40 cells in the presence of quinolones for 5 min, chromosomal DNA was isolated using lysozyme and sodium dodecyl sulfate. The extent of cleavable complex formation was determined by measuring the amount of [α-^{32}P]dCTP incorporated into broken chromosomal DNA ends in an end-filling reaction. The average standard deviations of DNA cleavage were 61, 144, 196, and 269 cpm at the concentrations 0.01, 0.1, 1, and 10 μg/ml of quinolones, respectively.

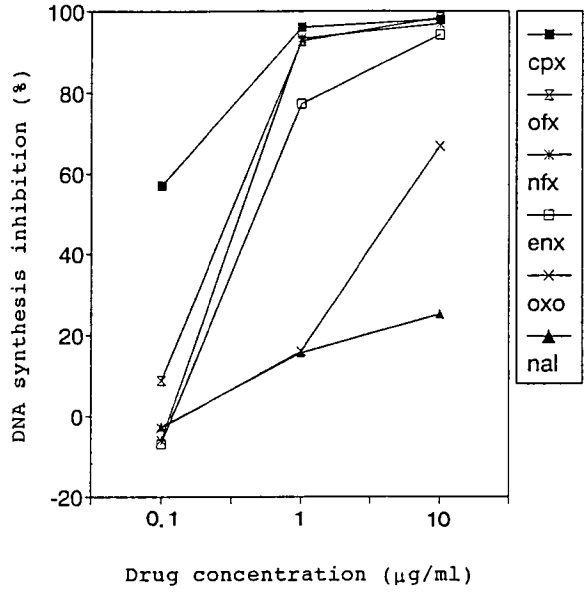

Fig. 3. Inhibition of DNA synthesis in *E. coli* PB40 at various concentrations of quinolones. DNA synthesis was quantified by measuring the amount of [α-^{3}H]thymidine incorporated into newly synthesized DNA after 5 min of pre-exposure to quinolones. The average standard deviation was 2.7 %.

DNA synthesis had a fairly high correlation coefficient of 0.9284 with percent nonviability, but it was slightly

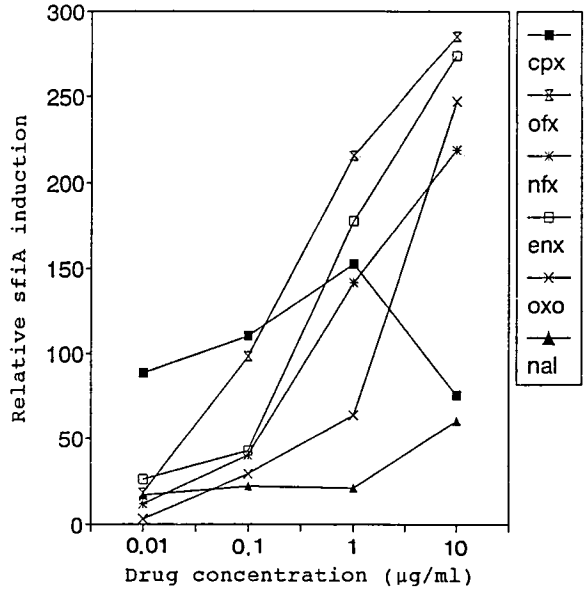

Fig. 4. *sfiA* gene induction in *E. coli* PB40 at various concentrations of quinolones. *E. coli* PB40 cells were incubated with quinolones for 30 min, then permeabilized to measure induction of SOS response. Induction was represented by the activity of β-galactosidase expressed under the control of the *sfiA* promoter. The average standard deviations of relative *sfiA* induction were 2.8, 7.8, 14.7, and 16.4 at the concentrations 0.01, 0.1, 1, and 10 μg/ml of quinolones, respectively.

lower than the correlation coefficient between chromosomal DNA cleavage and percent nonviability. *sfiA* gene induction had the lowest correlation coefficient of 0.8482 with percent nonviability, indicating that SOS response is probably not directly involved in the cell killing pathway. Walters *et al.* (1989) demonstrated that mutations in SOS reponse genes affected the cell killing kinetics of quinolones differently where, depending on mutated genes and quinolones, lack of SOS response either protected cells against quinolones or made cells more susceptible to quinolones. These results coincide with the relatively weak correlation between induction of SOS response and percent nonviability in this study. It may be argued that the weak correlation partly resulted from the disruption of the *sfiA* gene in the *E. coli* PB40 strain. Although this possibility can not be excluded, the effects of the gene disruption is thought to have been complemented or lessned by the *sfiC* gene playing almost the same role in SOS reponse. Piddock *et al.* (1990) demonstrated an excellent correlation between the concentration of quinolones required to inhibit DNA synthesis by 50%, the minimum inhibitory concentration, the maximum *recA*-inducing concentration, and the optimum bactericidal concentration.

The immediate cellular reactions which occur due to quinolone uptake are cleavable complex formation and a gradual decrease in negative DNA superhelicity.

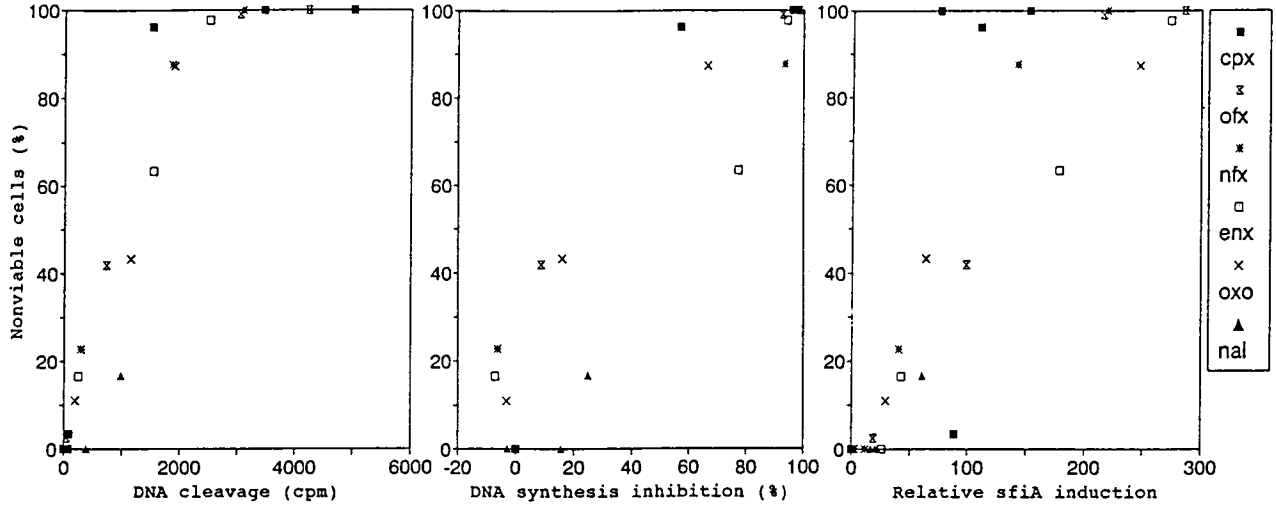

Fig. 5. Correlation of the bactericidal activity of quinolones with cellular reactions in *E. coli* PB40 caused by quinolones. Correlations were made between data points corresponding to the identical drug at the same concentrations of Figs. 1 to 4. Panel A: bactericidal activity vs. cleavable complex formation, Y=0.0446X−1.1788, correlation coeff. 0.9498. Panel B:7bactericidal activity vs. inhibition of DNA synthesis, Y=0.8574X+16.5502, correlation coeff. 0.9284. Panel C: bactericidal activity vs. induction of SOS response, Y=0.4070 X+3.9716, correlation coeff. 0.8482.

The cleavable complex and decreased negative DNA superhelicity cause inhibition of DNA synthesis through blockage of DNA replication forks and inefficient initiation of DNA synthesis, respectively. SOS response is induced by the cleavable complex where single-stranded regions may be generated through collision with DNA replication forks, or by the action of the RecDEF complex. Our results suggest the possibility that both chromosomal DNA cleavage and inhibition of DNA synthesis are in the quinolone-induced cell death pathway. On the other hand, SOS response is not considered to be a crucial factor in the pathway. Although inhibition of DNA synthesis is an important factor leading to cell death, it is not alone a sufficient condition since the bactericidal activity of quinolones and not inhibition of DNA synthesis is greatly enhanced by ongoing protein synthesis (Deitz *et al.*, 1966; Piddock *et al.*, 1990). Cleavable complex formation showed a better correlation with bactericidal activity than inhibition of DNA synthesis, implying that the cleavable complex contributes to cell death not only through inhibition of DNA synthesis, but also through another pathway. One subsequent process of cleavable complex formation is collision of the complex with replication forks, which may produce irreversably broken DNA ends. Irreversible DNA breaks were observed when human DNA topoisomerase I and its inhibitor camptothecin were present in an *in vitro* SV40 DNA replication system (Hsiang *et al.*, 1989; Tsao *et al.*, 1993). The irreversibly broken ends may initiate an unknown celluar reaction leading to cell death. It was demonstrated in Saccharomyces cerevisiae and mammalian cells that the cause of cell death by DNA topoisomerase II inhibitors is not the inhibition of DNA topoisomerase II activity but DNA damage due to cleavable complex formation (Davies *et al.*, 1988; D'Arpa *et al.*, 1990; Nitiss *et al.*, 1992). The results of this study also suggest that cleavable complex formation by quinolones is the main cause of cell death, and an unknown subsequent process(es) as well as inhibition of DNA synthesis are involved in the cell-killing pathway.

Acknowledgement

This work was supported by a grant from the Ministry of Science and Technology of the Republic of Korea.

References

Bernard, P. and Couturier, M. (1992) *J. Mol. Biol.* **226**, 735.
D'Arpa, P., Beardmore, C. and Liu, L. F. (1990) *Cancer. Res.* **50**, 6919.
Davies, S. M., Robson, C. N., Davies, S. L. and Hickson, I. D. (1988) *J. Biol. Chem.* **263**, 17724.
Deitz, W. H., Cook, T. M. and Goss, W. A. (1966) *J. Bacteriol.* **91**, 768.
Dinardo, S., Voelkel, K. and Sternglanz, R. (1984) *Proc. Natl. Acad. Sci.* USA **81**, 2616.
Drlica, K. and Franco, R. J. (1988) *Biochemistry.* **27**, 2253.
Gellert, M. (1981) *Annu. Rev. Biochem.* **50**, 879.
Hooper, D. C. (1993) *Drugs* 45, Suppl. 3, 8.
Hooper, D. C. and Wolfson, J. S. (1988) *Rev. Infectious Diseases* **10**, Suppl. 1, S14.
Horowitz, D. S. and Wang, J. C. (1987) *J. Biol. Chem.* **262**, 5339.

Hsiang, Y. H., Lihou, M. G. and Liu, L. F. (1989) *Cancer. Res.* **49**, 5077.

Huisman, O. and D'Ari, R. (1981) *Nature* **290**, 797.

Huisman, O. and D'Ari, R. (1983) *J. Bacteriol.* **153**, 169.

Huisman, O., D'Ari, R. and Gottesman, S. (1984) *Proc. Natl. Acad. Sci.* USA **81**, 4490.

Lockshon, D. and Morris, D. R. (1985) *J. Mol Biol.* **181**, 63.

Maguin, E., Lutkenhaus, J. and D'Ari, R. (1986) *J. Bacteriol.* **166**, 733.

Miller, J. H. (1972) *Experiments in Molecular Genetics*, pp. 352-356, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York.

Neu, H. C. (1988) *Medical Clinics of North America.* **72**, 623.

Nitiss, J. L., Liu, Y.-X., Harbury, P., Jannatipour, M., Wasserman, R. and Wang, J. C. (1992) *Cancer Res.* **52**, 4467.

Phillips, I., Culebras, E., Moreno, F. and Baquero, F. (1987) *J. Antimicro. Chemother.* **20**, 631.

Piddock, L. J. V., Walters, R. N. and Diver, J. M. (1990) *Antimicro. Agents Chemother.* **34**, 2331.

Shen, L. L. and Pernet, A. G. (1985) *Proc. Natl. Acad. Sci.* USA **82**, 307.

Shen, L. L., Baranowski, J. and Pernet, A. G. (1989) *Biochemistry* **28**, 3879.

Tsao, Y.-P., Russo, A., Nyamuswa, G., Silber, R. and Liu, L. F. (1993) *Cancer Res.* **53**, 5908.

Uemura, T., Ohkura, H., Adachi, Y., Morino, K., Shiozaki, K. and Yanagida, M. (1987) *Cell* **50**, 917.

Walker, G. C. (1984) *Microbiol. Rev.* **48**, 60.

Walters, R. N., Piddock, L. J. V. and Wise, R. (1989) *J. Antimicro. Chemother.* **24**, 863.

Wang, J. C. (1985) *Annu. Rev. Biochem.* **54**, 665.

Wang, J. C. (1987) *Biochim. Biophys. Acta* **909**, 1.

Wu, H. Y., Shyy, S., Wang, J. C. and Liu, L. F. (1988) *Cell* **53**, 433.

Yang, L., Wold, M. S., Li, J. J., Kelly, T. J. and Liu, L. F. (1987) *Proc. Natl. Acad. Sci.* USA **84**, 950.

Zweerink, M. M. and Edison, A. (1986) *Antimicro. Agents. Chemother.* **29**, 598.