RESEARCH COMMUNICATION

Comparative Evaluation of Antiproliferative Activity and Induction of Apoptosis by some Fluoroquinolones with a Human Non-small Cell Lung Cancer Cell Line in Culture

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Abstract

Lung cancer is the leading cause of cancer- related death in the world today. Since the effective management of drug resistant lung cancer, and particularly non-small cell lung carcinomas is a major problem, attempts need to be made to identify new potential anticancer drugs that can kill non-small cell lung cancer cells efficiently. In the present study, a human non-small cell lung carcinoma NCI-H460 cell line was used to evaluate the antiproliferative activity of Fluoroquinolones like Enoxacin, Norfloxacin, Ciprofloxacin and Levofloxacin. As determined by Sulphorodhamine B assay (SRB assay), all Fluoroquinolones caused cellular growth inhibition in a concentration and time-dependent manner. Enoxacin was found to be the most effective Fluoroquinolone followed by Norfloxacin, Ciprofloxacin and Levofloxacin. Growth inhibitory effects were also found to be independent of the concentrations of serum growth factors in culture medium or variation of initial cell seeding density and proved to be irreversible in nature. Appearance of rounded cells with altered morphology and cell surface blebbing indicated cell killing by apoptosis. Cell shrinkage, nuclear condensation & fragmentation, and cytoplasmic blebbing as indicated by MGG staining confirmed this to be the case. Thus, this investigation clearly demonstrated that the NCI-H460 human non-small cell lung carcinoma cell line is highly sensitive to Fluoroquinolone treatment. The Fluoroquinolones used in this study which are clinically used as antibacterial agents, can also inhibit tumor cell growth suggesting their potential use in a strategy for cancer treatment which might help in controlling cancer.

Key Words: Fluoroquinolones - NCI-H460 cell line - apoptosis - growth inhibition

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Introduction

Lung cancer is the most frequently diagnosed malignancy throughout the world and its global incidence is increasing primarily due to increase in cigarette smoking habits in the developing countries (Tormanen, 1999). World wide more than 1.3 million lung cancer deaths were expected in the year 2001 (Averbuch, 2002) and this continues to rise. Despite improved methods of lung cancer detection and technical advances in treatment modalities, it is found that the efficacy of different anticancer agents for treating advanced stages of lung cancer is limited. It has also been reported that non-small cell lung carcinoma (NSCLC) shows relative resistance to anticancer drug and radiation treatment in contrast to the initial responsiveness of small cell lung carcinoma (SCLC) to the same type of intervention (Joseph et al., 2002). Thus, search for new drugs with potential anti tumor activity in treating lung cancer particularly NSCLC is needed.

Fluoroquinolones are synthetic, relatively non-toxic, broad-spectrum antibiotics which inhibit the bacterial DNA gyrase / type II DNA topoisomerase in case of mammalian cells. The enzyme is responsible for supercoiling, transcription, replication and chromosomal separation of prokaryotic DNA (Chen and Liu, 1994). These antibiotics are used clinically for the treatment of wide variety of bacterial infections and can be administered orally at high doses and are found to reach concentrations far above those of the serum in solid tissues like lung (Herold et al., 2002; Sharma et al., 1994). Ciprofloxacin, a commonly used fluoroquinolone antibiotic has recently been shown to have antiproliferative and apoptotic activity against several cancer

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cells (Aranha et al., 2000; Herold et al., 2002). Several other Fluoroquinolones like Ofloxacin, Levofloxacin and Fleroxacin to some extent have also been shown to inhibit growth of transitional bladder carcinoma cell lines (Seay et al., 1996; Yamakuchi et al., 1997; Ebisuno et al., 1997).

Considering the growth inhibitory potentiality of some Fluoroquinolones on certain neoplastic cells and due to lack of adequate sensitivity of non-small cell lung carcinoma to anticancer agents, attempt has been made for the first time to evaluate the antiproliferative and apoptotic activity of some Fluoroquinolone antibiotics like Enoxacin, Norfloxacin, Ciprofloxacin and Levofloxacin on a human non small-cell lung carcinoma cell, NCI-H460 in culture.

Materials and Methods

Cell Culture:

NCI-H460, human non-small-cell lung cancer cell line was obtained from National Cancer Institute, USA. The cells were maintained in RPMI 1640 medium (GIBCO, USA) containing 5% fetal bovine serum (FBS), supplemented with additional glutamine (0.03%) and 50 µg/ml gentamycin. Cells were grown in plastic tissue culture flasks (NUNC) at 37°C, 5% CO_2 and 95% air. Cells from exponentially growing culture were used for all experiments. All the experiments were repeated three times.

Drug:

Enoxacin and Norfloxacin were purchased from Sigma Chemical Co., USA. Ciprofloxacin and Levofloxacin were obtained as gift from Ranbaxy Laboratories (India) Limited. Stock solution of Fluoroquinolones was initially prepared in dimethyl sulfoxide (DMSO) and final concentrations of 10 -200µg/ml were produced by diluting drug stocks in complete growth medium. In all cases control experiments were carried out in medium containing 0.25 % DMSO.

Cell Proliferation Assay:

To assess Fluoroquinolone induced growth inhibition Sulphorodhamine B assay (SRB assay) was performed as described with some modifications (Monks et al., 1991). Briefly NCI-H460 cells were seeded in flat-bottomed 96-well microtitre plates at a density of 2500 cells /well. Cells were allowed to adhere for 24 hours and were then exposed to different concentrations of Enoxacin (10µg/ml-50 µg/ml), Norfloxacin (10µg/ml- 50 µg/ml), Ciprofloxacin (10 µg/ml -100µg/ml) and Levofloxacin (10 µg/ml-200µg/ml) for 5 days. Drug treated plates were fixed at an interval of 24 hours in situ by cold 50 % trichloroacetic acid (TCA) at a final concentration of 10 % and incubated for 60 min at 4°C. The plates were washed with deionized water, dried and stained with 0.4% SRB in 1% acetic acid, incubated for 10 min at room temperature. Unbound stain was removed by washing with 1% acetic acid and air dried. Bound stain was solublized with 10 mM trizma base and optical densities of all the wells were read on an automated microplate reader at a wavelength of 515nm.

Morphology:

For assessment of morphological changes, 2 X 10^4 cells were seeded in a 35mm petridish (NUNC) containing a coverslip and 1.5ml growth medium. After 24 hours of plating, plates were treated with Enoxacin (20 µg/ml), Norfloxacin (40µg/ml), Ciprofloxacin (40µg/ml) and Levofloxacin (100 µg/ml)separately. Finally after 72 hours of incubation, cells were fixed in Bouin's fluid and stained with haematoxylene-eosin (HE) and mounted in D.P.X.

Assessment of Apoptotic Cells:

Morphological assessment of the apoptotic cells was performed using the May-Grunwald-Giemsa (MGG) staining method [Tolis et al., 1999]. Cells were treated separately with Enoxacin (20 µg/ml), Norfloxacin (40µg/ml), Ciprofloxacin (40µg/ml) and Levofloxacin (100 µg/ml). At 24, 48, 72 and 96 hours cells were harvested and cytospins were prepared. The slides were dried overnight and stained with MGG stain. The morphology of cells was examined under light microscope. Apoptotic cells were identified by criteria previously described (Kerr et al., 1994). An apoptotic index (AI) was determined as the percentage of apoptotic cells from at least 400 counted cells.

Irreversibility of Cell Growth Inhibition:

Irreversibility of Fluoroquinolone- induced growth inhibition was studied by plating 6 X 10^4 cells per 35mm petridish. After 24 hours, each of plates was treated separately with Enoxacin (20 µg/ml), Norfloxacin (40µg/ml), Ciprofloxacin (40µg/ml) and Levofloxacin (100 µg/ml). After 72 hours of incubation, media of the control culture plates and half of the Fluoroquinolone treated plates were replaced with fresh growth medium only and in the other half Fluoroquinolone treatment was continued for another 72 hours. Cells were counted by using hemocytometer at an interval of 24 hours employing trypan blue dye exclusion technique. Population doubling time (Patterson, 1979) and saturation densities of control and treated cultures were calculated.

Role of Serum:

The role of different concentrations of serum (FBS) in the growth medium in Fluoroquinolone-induced growth inhibition were assessed by plating 6 X 10^4 cells per 35mm petridish. After 24 hours, Enoxacin (20 µg/ml), Norfloxacin (40 µg/ml), Ciprofloxacin (40 µg/ml) and Levofloxacin (100 µg/ml) were added separately to the medium supplemented with 0.5 % - 10 % FBS. Media renewal in drug treated plates as well as in control plates were done on 3rd day and the cells were counted on 5th day as described above.

Role of Cell Seeding Density:

Fluoroquinolone-induced growth inhibition on different cell densities of NCI-H460 cells were studied by seeding cells at different initial cell densities (1 X 10^4 – 10 X 10^4) per 35 mm culture dishes. Next day the cells were treated separately with Enoxacin (20 µg/ml), Norfloxacin (40µg/

ml), Ciprofloxacin (40μg/ml) and Levofloxacin (100 μg/ml). Media renewal and cell counting on 5th day were done as described above.

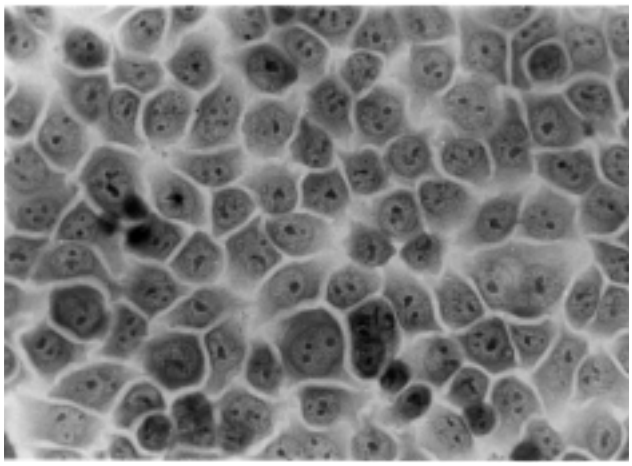
Results

Morphological Changes:

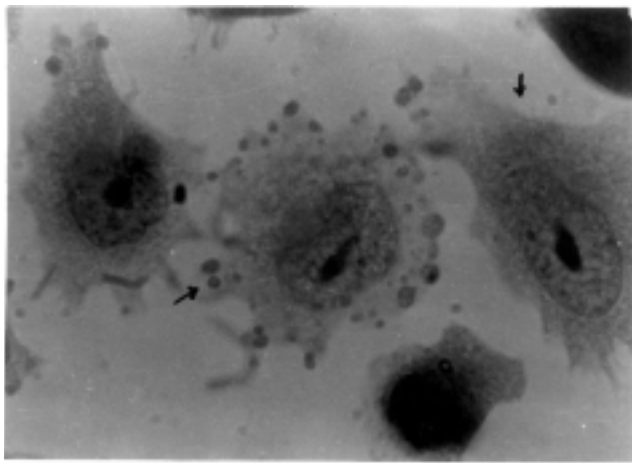
Fluoroquinolone induced significant morphological alterations in NCI-H460 cells in culture. Control cells were regular in shape and size, had eccentric nucleus with multiple nucleoli and relatively small amount of cytoplasm (Fig.1A). Cells treated with Enoxacin (20 μg/ml), Norfloxacin (40 μg/ml), Ciprofloxacin (40 μg/ml) and Levofloxacin (100 μg/ml) showed almost similar type of morphological alterations. Majority of the cells was rounded in appearance and showed cell surface blebbing, an early feature of apoptosis (Fig1 B. i). Some cells became irregular in shape and size with altered nuclear: cytoplasmic ratios, elongated cytoplasmic processes and had relatively flattened appearance (Fig1B. ii) indicating that these drugs induced some changes in cell surface associated with the adherence to the substratum. Appearance of multinucleated giant cells suggesting inhibition of cytokinesis was also observed in few cells (Fig 1B.iii).

Induction of Apoptosis:

MGG staining of cytospin preparations of the NCI-H460 cells treated separately with Enoxacin (20 μg/ml), Norfloxacin (40 μg/ml), Ciprofloxacin (40 μg/ml) and Levofloxacin (100 μg/ml) showed distinct morphological features for apoptosis. Compared to untreated control cells (Fig 2 A), majority of the cells showed membrane irregularity, cell shrinkage, cytoplasmic blebbing, nuclear condensation and fragmentation (Fig 2 B, C), and finally cellular fragments (Fig 2 D) were also observed in some cells. Detailed analysis of the AI (Fig 3) indicated that the percentage of apoptotic cells in all cases increased in a time dependent manner from 12.56% to 43.50% in case of Enoxacin, 11.44% to 32.69% in case of Norfloxacin, 16.71%



1 A



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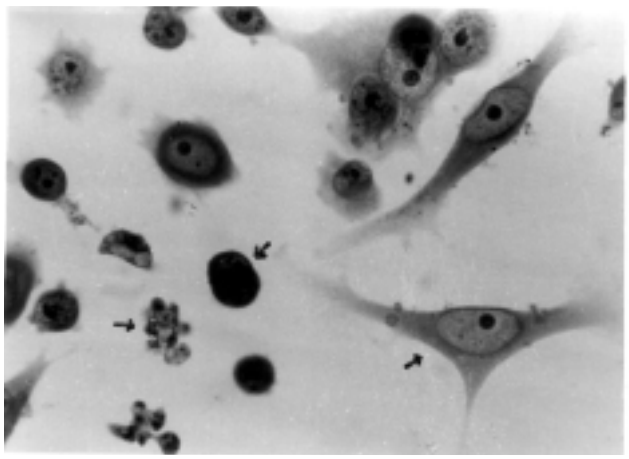

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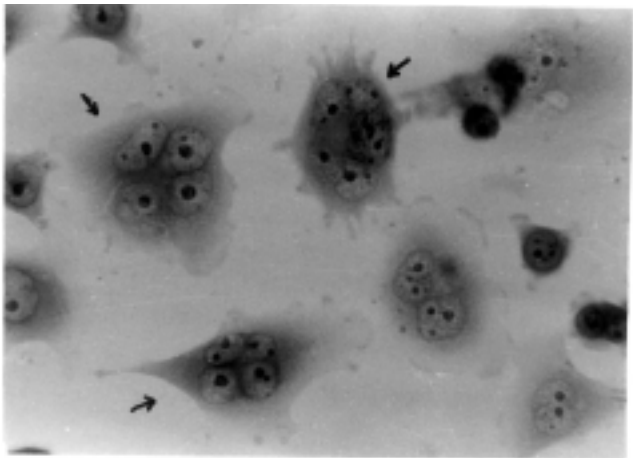

1B (iii)

Figure 1. Fluoroquinolone-induced Morphological Alterations in NCI-H460 Cell after 3 days of Exposure [A] Control culture: regular in shape and size with eccentric nucleus. (400X) [B] Enoxacin (20 μg/ml) treated culture. (i)Appearance of rounded cells, cell surface blebbing and elongated cytoplasmic processes (400X). (ii) Cells flattened in appearance (1000X). (iii) Appearance of multinucleated giant cells (400 X).

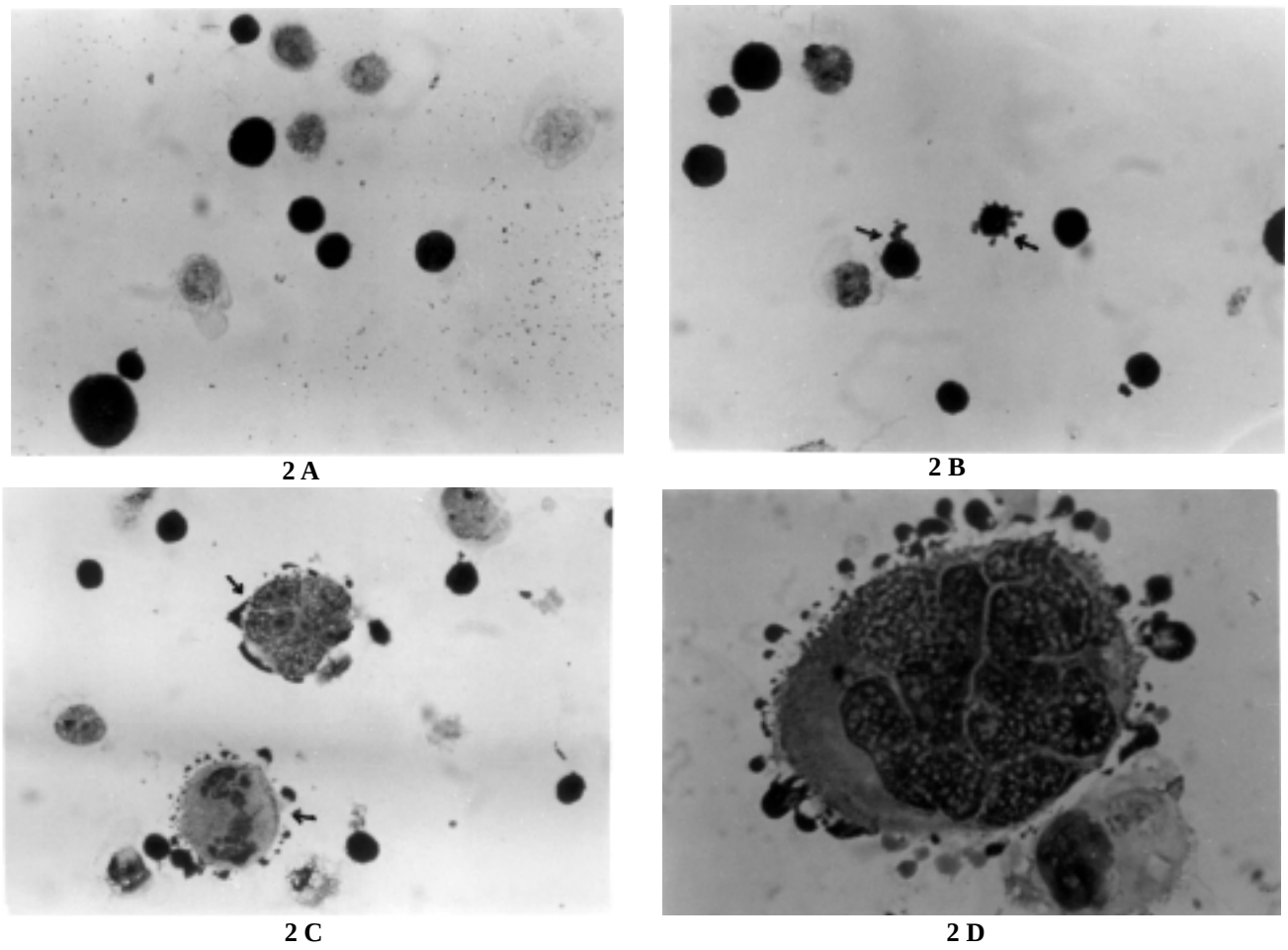

Figure 2. Apoptotic Morphology upon Fluoroquinolone Treatment (MGG staining) [A] Untreated control cultures (400X) [B] Enoxacin (20 μg/ml) treated culture: cell shrinkage, membrane irregularity (400X). [C] Chromatin condensation and nuclear fragmentation (400X). [D] Formation of apoptotic bodies (1000X).

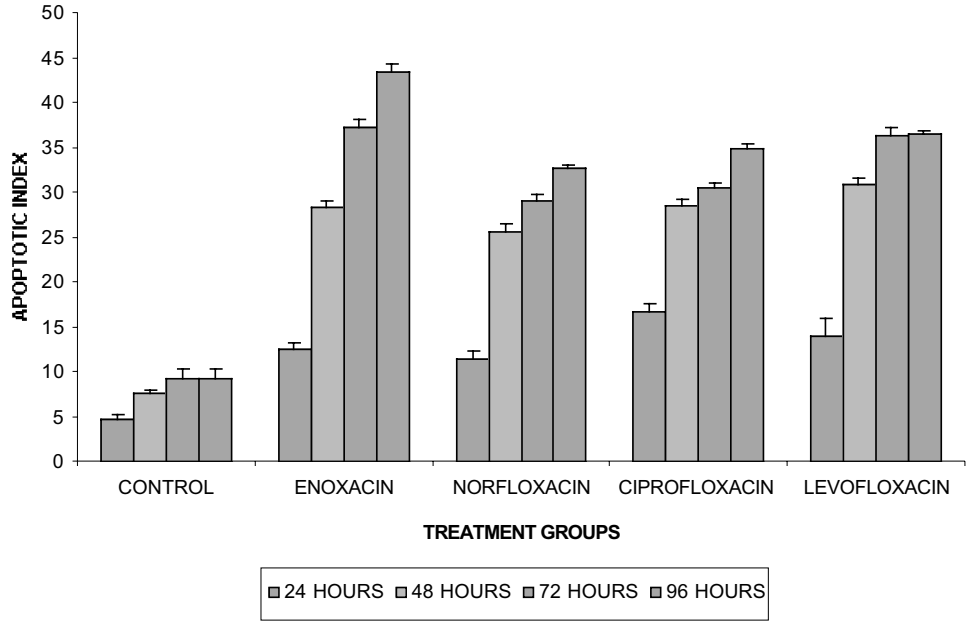

Figure 3. Histogram of Apoptotic Index (AI) of Fluoroquinolone Treated NCI-H460 Cells after MGG Staining. Enoxacin (20 μg/ml), Norfloxacin (40 μg/ml), Ciprofloxacin (40 μg/ml) and Levofloxacin (100 μg/ml) were used. Each point is the mean of triplicate culture, bars represent ± S. E. where bars are not shown, and they are equal to the sizes of symbols. Similar results were obtained in three independent experiments

to 34.88% in case of Ciprofloxacin and 14.08% to 36.60% in case of Levofloxacin from 24 to 96 hours of drug exposure.

Dose Dependent Effect of Fluoroquinolone-induced Growth Inhibition:

The effects of various concentrations of Enoxacin, Norfloxacin, Ciprofloxacin and Levofloxacin on the proliferation of NCI-H460 cells at different time periods are shown in Fig 4. A, B, C and D. Detailed analysis of the results indicates that the percentage of growth inhibition in all cases increased in a concentration and time dependent manner. As determined by SRB assay, after 5 days of drug exposure Enoxacin inhibited cell proliferation significantly from 78.71%-96.39%, Norfloxacin from 68.73%-82.72%, Ciprofloxacin from 64.83%-100.0% and Levofloxacin from 64.68%-80.25% at drug concentrations ranging from 10-50 μg/ml, 20-50 μg/ml, 20-100 μg/ml and 80-200 μg/ml respectively. Fifty percent growth inhibitory concentrations (GI 50) after 72 hours of drug exposure were found to be 11.5 μg/ml, 27.5 μg/ml, 19.5 μg/ml and 55.0 μg/ml for Enoxacin, Norfloxacin, Ciprofloxacin and Levofloxacin respectively. From GI 50 values it is evident that Enoxacin is the most effective Fluoroquinolone followed by Ciprofloxacin and Norfloxacin. Levofloxacin is least effective as compared to other Fluoroquinolones.

Irreversibility of Fluoroquinolone-induced Growth Inhibition:

Anchorage dependent growth kinetics of NCI-H460 cells in presence and in absence of Enoxacin (20 μg/ml), Norfloxacin (40 μg/ml), Ciprofloxacin (40 μg/ml) and Levofloxacin (100 μg/ml) are shown in figure 5. A, B, C and D. The results show that the growth inhibition induced by Fluoroquinolones is time dependent. From the figure it is evident that the cells in the untreated cultures grew significantly upto 5 days beyond which they reached at the plateau phase. But all Fluoroquinolone treated cultures grew slowly unto 3 days and the percentage of growth inhibition

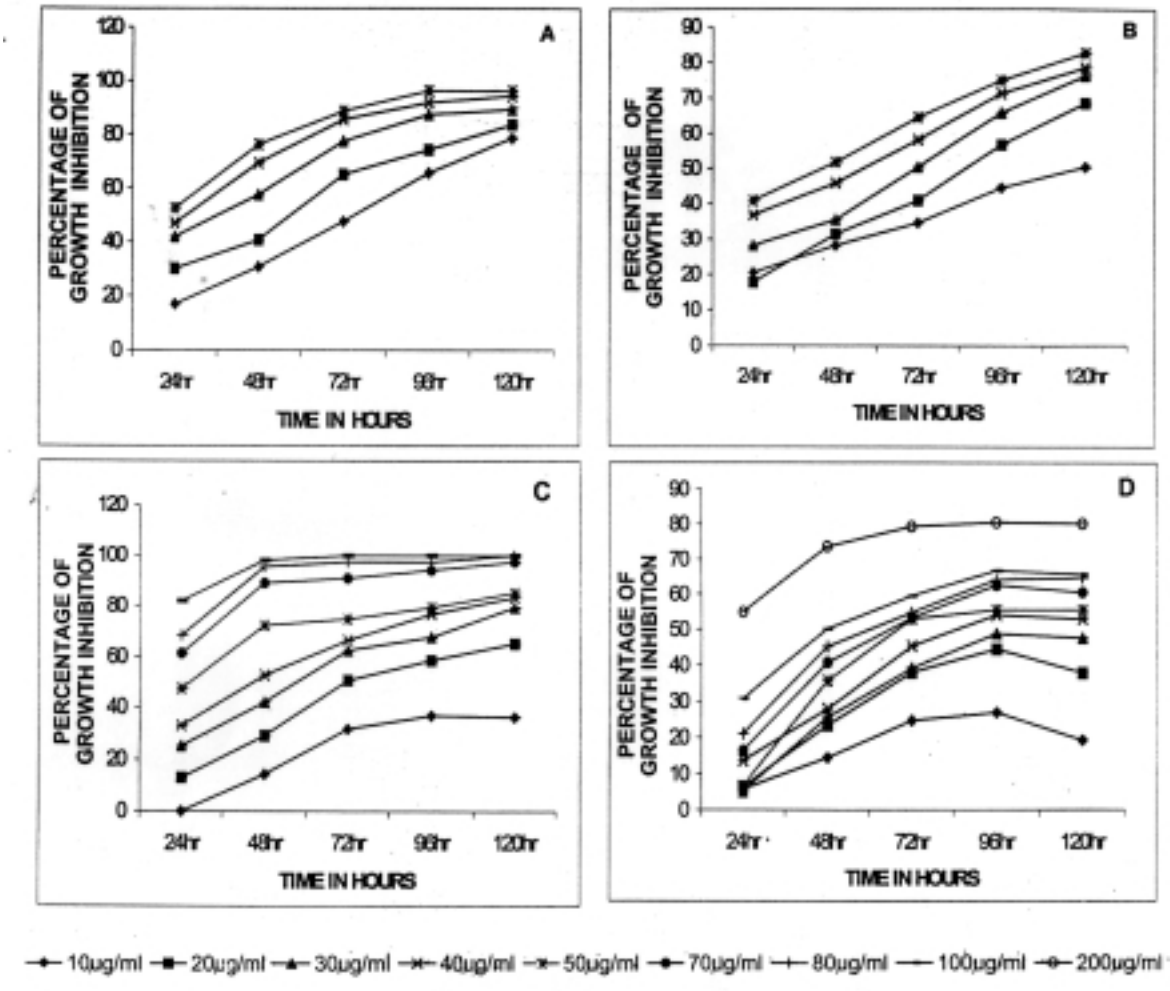

Figure 4. NCI-H460 Cell Growth Inhibition Induced by Fluoroquinolones. A. Enoxacin (10-50 μg/ml), B. Norfloxacin (10-50 μg/ml), C. Ciprofloxacin (10-100 μg/ml) and D. Levofloxacin (10-200 μg/ml). Each point is the mean of triplicate culture, bars represent ± S. E. where bars are not shown, and they are equal to the sizes of symbols. Similar results were obtained in three independent experiments.

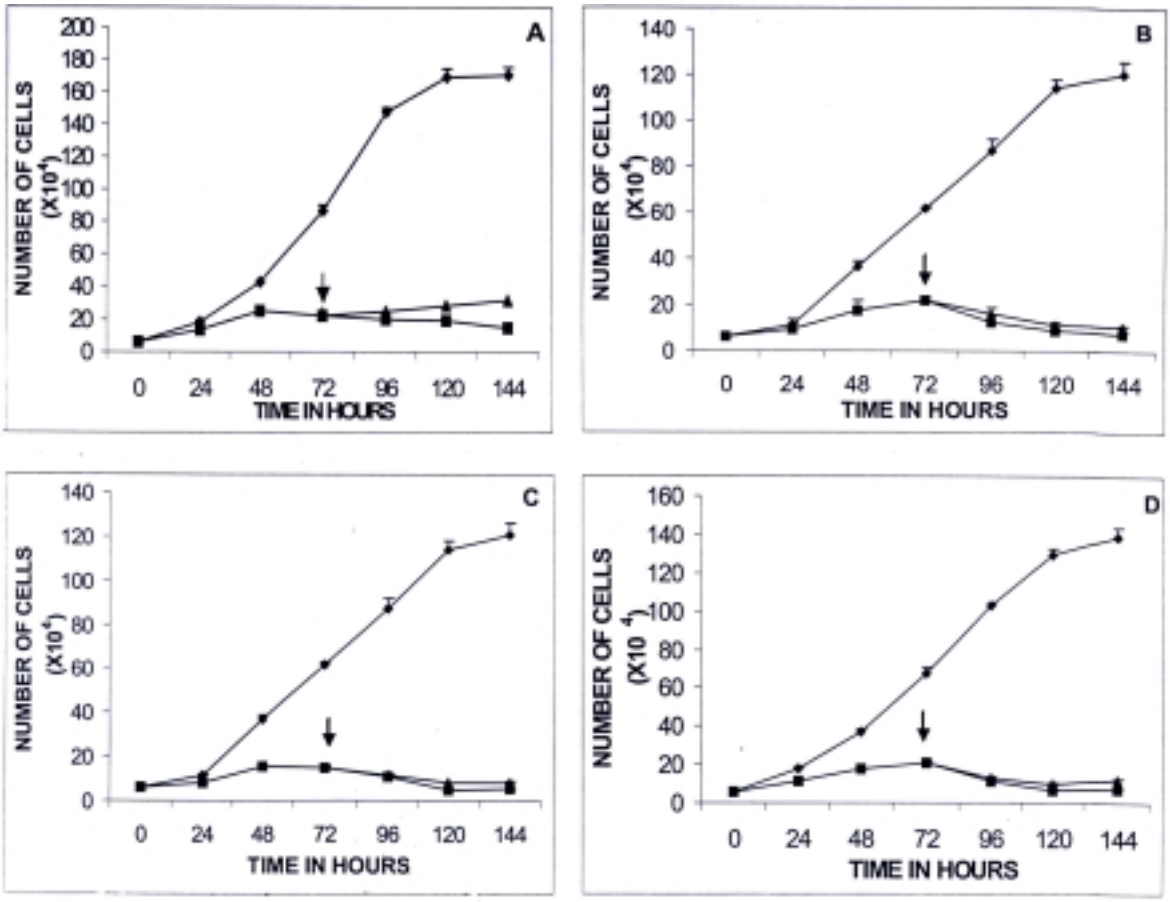

Figure 5. Growth Kinetics and Irreversibility of Fluoroquinolone Induced Growth Inhibition of NCI-H460 Cells. Assays were Performed as Described in Materials and Methods. A. Enoxacin (20μg/ml), B. Norfloxacin (40μg/ml), C. Ciprofloxacin (40 μg/ml) and D. Levofloxacin (100 μg/ml). (Arrow) Point of fluoroquinolone withdrawal from the medium, (Circles) Untreated NCI-H460 cells, (Squares) Fluoroquinolone treated cells and (triangles) fluoroquinolone withdrawn from the medium. Each point is the mean of cell numbers from triplicate plates. Bars represent ± S.E. Where bars are not shown they are equal to the sizes of the symbols. Similar results were obtained in 3 independent experiments.

was found to be 74.29%, 65.03%, 76.50% & 68.75% and when the drug treatment was continued for another 3 days the cell number started to decline gradually and the percentage of growth inhibition increased to 91.21%, 93.58%, 95.78% and 94.55% after Enoxacin, Norfloxacin, Ciprofloxacin and Levofloxacin treatment respectively. Whereas when the cultures exposed to all Fluoroquinolones for 72 hrs were incubated in drug free medium for another 72hrs, the percentage of growth inhibition was found to be 81.50 %, 91.09 %, 93.39% and 91.34% respectively. As the percentage of growth inhibition in all cases even after drug withdrawal remained significantly high, the Fluoroquinolone-induced growth inhibition can be stated as to be irreversible in nature. It was also observed that the minimum population doubling time (17.88 hr) in control cultures increased to 23.27 hr, 31.0 hr, 35.98 hr and 30.63 hr after Enoxacin, Norfloxacin, Ciprofloxacin and Levofloxacin treatment respectively. The results also indicate that in comparison to untreated cultures there was 11.85-fold, 15.58-fold, 23.8-fold and 18.34-fold reduction in saturation density after 6 days of Enoxacin, Norfloxacin, Ciprofloxacin and Levofloxacin treatment respectively.

Serum Requirement of Fluoroquinolone-treated Cells:

Effects of various concentrations of serum in the growth media on the cellular proliferation on Fluoroquinolone-treated and untreated NCI-H460 cells are shown in Table 1. At all serum concentrations (0.5%- 10.0 %) tested, growth of Fluoroquinolone -treated cultures were significantly retarded as compared to untreated cultures. It was also observed that at all serum concentrations the percentage of growth inhibition of Fluoroquinolone-treated cells almost remain unaltered and were found to be approximately 90% in case of Enoxacin, 93% in case of Norfloxacin and Ciprofloxacin and 94% in case of Levofloxacin. The results show that Fluoroquinolone-induced growth inhibition is independent of variation of concentration of the serum in the culture medium.

Fluoroquinolone-induced Growth Inhibition at Different Cell Densities:

Fluoroquinolone-induced growth inhibition of NCI-H460 cells seeded at different cell densities is shown in Table 2. The results show that the magnitude of growth of cells was significantly retarded at all cell densities tested. There

Table 1. Effects of Serum Concentrations on NCI-H460 Cell Growth Inhibition after 5 Days of Treatment with Enoxacin, Norfloxacin, Ciprofloxacin and Levofloxacin (results are mean ± S. D.)

Serum Concentration(%)	No. of cells(x 10^4)				
	Control (0.25%DMSO)	Enoxacin (20 μg/ml)	Norfloxacin (40 μg/ml)	Ciprofloxacin (40 μg/ml)	Levofloxacin (100 μg/ml)
0.5	81.88 ± 3.72	8.07 ± 0.26 (90.14 ± 0.85)	7.36 ± 0.58 (91.01 ± 1.04)	5.82 ± 0.27 (92.89 ± 1.16)	5.03 ± 0.61 (93.85 ± 0.76)
1.0	93.92 ± 0.58	9.04 ± 0.21 (90.38 ± 0.21)	7.55 ± 0.59 (91.96 ± 0.70)	4.87 ± 0.26 (94.81 ± 0.54)	5.17 ± 0.29 (94.53 ± 0.38)
3.0	111.63 ± 3.64	10.77 ± 0.21 (90.35 ± 0.12)	7.96 ± 0.28 (92.86 ± 0.35)	6.56 ± 0.40 (94.12 ± 0.71)	5.50 ± 0.41 (95.07 ± 0.31)
5.0	119.95 ± 3.15	11.07 ± 0.22 (90.77 ± 0.58)	7.80 ± 0.27 (93.47 ± 0.23)	7.30 ± 0.31 (93.91 ± 0.53)	6.10 ± 0.31 (94.92 ± 0.34)
8.0	126.94 ± 1.33	12.19 ± 0.62 (90.39 ± 0.46)	8.81 ± 0.45 (93.06 ± 0.41)	8.92 ± 0.17 (92.97 ±0.27)	7.27 ± 0.24 (94.26 ± 0.19)
10.0	137.75 ± 0.63	13.75 ± 0.85 (90.02 ± 0.70)	8.41 ± 0.13 (93.89 ± 0.61)	10.35 ± 0.23 (92.48 ± 0.16)	7.40 ± 0.36 (94.63 ± 0.16)

Figures in parenthesis show percentage of growth inhibition.

was a slight decrease of percentage of growth inhibition from 95.1 % to 92.17 % in case of Enoxacin, 94.07 % to 88.97 % in case of Norfloxacin and 96.89% to 90.89% in case of Levofloxacin as the initial cell seeding density increased from 1 X 10^4 to 10 X 10^4 cells per dish. This decrease of growth inhibition was apparent unto 4 X 10^4 cells and beyond that almost no such decrease was noticed in the above cases. Whereas in case of Ciprofloxacin the percentage of inhibition ranged from 95.53 % to 94.94 % and was almost unaltered with increase of cell densities. Thus the results clearly demonstrate that Fluoroquinolone-induced growth inhibition is independent of cell seeding densities.

Discussion

Our results demonstrate that the treatment of human non small–cell lung carcinoma cells with different Fluoroquinolone antibiotics like Enoxacin, Norfloxacin, Ciprofloxacin and Levofloxacin induced morphological alterations, apoptotic cell death, inhibited cellular proliferation, increased population doubling time and reduced saturation density. In addition it was also observed that Fluoroquinolone-induced growth inhibition is independent of concentration of the serum in the growth medium and different initial cell seeding densities.

Reports from several laboratories have shown that various tumor cells undergo morphological changes following exposure to Fluoroquinolone antibiotics in culture (Arahna et al., 2000; Ebisuno et al., 1997; Herold et al., 2002). All the four Fluoroquinolones used in this study induced almost similar type of morphological alterations in NCI-H460 cells. Some cells became rounded, detached and showed cell membrane blebbing, a typical morphological change indicating initiation of apoptotic processes. Similar changes were reported in case of Ciprofloxacin treated tranisitional cell carcinoma of bladder and colorectal

Table 2. Effects of Cell Seeding Density onNCI-H460 Cell Growth Inhibition after 5 Days of Treatment with Enoxacin, Norfloxacin, Ciprofloxacin and Levofloxacin (results are mean ± S. D.)

Cell density Cells/ 35mm Plate	No. of cells (x 10^4)				
	Control (0.25% DMSO)	Enoxacin (20 μg/ml)	Norfloxacin (40 μg/ml)	Ciprofloxacin (40 μg/ml)	Levofloxacin (100 μg/ml)
1 X 10^4	19.56 ± 2.07	0.95 ± 0.41 (95.10 ± 0.80)	1.16 ± 0.07 (94.07 ± 1.69)	0.87 ± 0.09 (95.53 ± 0.29)	0.61 ± 0.03 (96.89 ± 0.80)
2 X10^4	84.63 ± 2.66	4.69 ± 0.22 (94.46 ± 0.21)	5.15 ± 0.27 (93.92 ± 0.98)	2.89 ± 0.30 (96.59 ± 0.33)	2.40 ± 0.13 (97.17 ± 0.52)
4 X 10^4	118.25 ± 5.04	10.03 ± 0.78 (91.44 ± 0.51)	14.43 ± 1.08 (87.79 ± 0.94)	6.55 ± 0.55 (94.36 ± 0.46)	5.58 ± 0.38 (95.28 ± 0.34)
6 X 10^4	146.63 ± 3.97	11.41 ± 0.51 (92.21 ± 0.51)	17.60 ± 2.13 (88.00 ± 1.52)	7.3 ± 0.30 (94.70 ± 0.59)	11.94 ± 0.33 (91.86 ± 0.37)
8 X 10^4	166.5 ± 3.54	12.47 ± 0.76 (92.51 ± 0.46)	19.81 ± 1.06 (88.10 ± 0.35)	8.28 ± 0.34 (95.03 ± 0.25)	14.14 ± 1.44 (91.51 ± 1.23)
10 X 10^4	183.38 ± 6.21	14.39 ± 0.63 (92.17 ± 0.23)	20.23 ± 0.48 (88.97 ± 0.45)	9.26 ± 0.18 (94.94 ± 0.19)	16.71 ± 1.11 (90.89 ± 0.65)

Figures in parenthesis show percentage of growth inhibition.

carcinoma (Arahna et al., 2000; Herold et al., 2002). In our study, appearance of multinucleated giant cells indicated inhibition of cytokinesis and change in the ploidy level of treated cells. Further studies are required to analyze the ploidy level of Fluoroquinolone treated NCI-H460 cell. Besides certain percentage of cells had relatively flattened appearance with altered nuclear: cytoplasmic ratios indicating that these Fluoroquinolones induced some change in the cell surface associated with the adherence to the substratum and treated cells tend to adhere strongly to the substratum, an opposite behaviour of the tumor cells which tend to attach loosely to the substratum both in vitro and in vivo systems (Mukherjee and Das, 1995). The morphological changes for initiation of apoptosis were further studied by performing MGG staining. Cell shrinkage, chromatin condensation, nuclear fragmentation and the formation of apoptotic bodies confirmed Fluoroquinolone mediated apoptotic cell death. Similar changes were also reported in case of Ciprofloxacin treated tranisitional cell carcinoma of bladder and colorectal carcinoma (Arahna et al., 2000; Herold et al., 2002) These differential morphological alterations suggest that the reduced growth of NCI-H460 cells could be attributable to the apoptotic cell death and inhibition of cytokinesis in addition to cell growth inhibition.

Initially it was reported that Fluoroquinolones only inhibited bacterial type II DNA gyrase but the influence of Fluoroquinolone on growth of certain eukaryotic cells was not known. Recently few reports documented the antiproliferative effects of some Fluoroquinolones like Ofloxacin, Levofloxacin, Fleroxacin and Ciprofloxacin on human trasitional cell carcinoma of bladder (Arahna et al., 2000; Ebisuno et al., 1997; Seay 1996; Yamakuchi et al., 1992) and colorectal carcinoma (Herold et al., 2002). In cell free system Hussy et al.1986, demonstrated the inhibitory activity of Fluoroquinolones against mammalian DNA topoisomerase I, topoisomerase II and DNA polymerase. They reported that Ciprofloxacin was the most potent Fluoroquinolone against these enzymes. Our results also clearly showed that all four Fluoroquinolones inhibited cell growth significantly both in concentration- and time-dependent manner as shown in some previous reports (Arahna et al., 2000; Herold et al., 2002; Yamakuchi et al., 1992). We showed for the first time that Enoxacin caused significant growth inhibition even at 20 mg/ml concentration compared to other Fluoroquinolones used in this study as well as in other studies reported earlier (Arahna et al., 2000; Ebisuno et al., 1997; Herold et al., 2002; Seay et al., 1996; Yamakuchi et al., 1992). Furthermore, lung cancer NCI-H460 cell was found to be more sensitive to Fluoroquinolone treatment as compared to other cell lines reported earlier (Arahna et al., 2000; Ebisuno et al., 1997; Herold et al., 2002; Seay et al., 1996; Yamakuchi et al., 1992). It has been reported that the chemosensitivity to topotecan and gemcitabine treatment of lung cancer cells depends on the p53 status of the cell (Tolis et al, 1999). Probably the presence of wild type p53 expression in NCI-H460 cells confers the sensitivity towards Fluoroquinolone treatment (Tolis et al, 1999). Our data also demonstrated that Fluoroquinolone induced increase in population doubling time and decrease of saturation density in NCI-H460 cells. These effects, which can be regarded as density dependent growth control, are similar to previous findings with transformed murine L-929 and Nllpy fibroblast cells (Dion et al., 1977; Patt et al., 1978) as well as in human brain tumor cells (Mukherjee and Das, 1995) after retinoic acid treatment. All these effects of Fluoroquinolones was found to be irreversible in nature, suggesting that after treatment with fluoroquinolone once the cells are programmed to die, they cannot respond to normal growth conditions.

It has been reported that the transformed cells usually have lower serum requirements compared to their normal counterpart (Jetten, 1981). Our results clearly demonstrated that the untreated non-small cell lung carcinoma cells are capable to grow nicely at very low concentrations of serum. But after Fluoroquinolone-treatment, the increased saturation density and decreased serum requirements which are associated with the transformed state of these untreated cells appears to have altered and as a result of which the Fluoroquinolone -treated NCI-H460 cells grew at a very low density even in presence of high concentration of serum. The drug-induced alterations in the serum requirement had been observed in case of glioblastoma (Mukherjee and Das, 1995), oral carcinoma (Sarkar and Das, 1997) and murine 3T6 cells (Jetten, 1981) after retinoic acid treatment. In this study it was observed that growth inhibitory effects of Fluoroquinolones are independent of serum concentrations and initial cell densities. Accumulating evidence indicate that Topoisomerase II levels of HeLa cell remained less affected with changing of serum concentration and cell density (Hwang and Hwang, 1994). It was also reported that nuclear Topoisomerase II level correlate with the sensitivity of the Topoisomerase II targeting drugs (Davis et al., 1988). Thus in our cell system the possibility of presence of almost same level of Topoisomerase II enzyme at different serum concentrations and cell seeding density may exist and as a consequence of these facts percentage of growth inhibition of this lung cancer cell almost remained unchanged at different serum concentrations and cell seeding densities after treatment with Topoisomerase II poisons like Enoxacin, Norfloxacin, Ciprofloxacin and Levofloxacin in culture. But further experiments have to be done to establish this.

Our data provide strong evidence about the growth inhibitory potentiality and apoptotic induction by these Fluoroquinolone antibiotics on human non-small cell lung carcinoma in culture while inadequate response to chemotherapeutic drugs is a major obstacle to the effective treatment of non-small cell lung cancer. The mechanism by which the Fluoroquinolones exert their growth inhibitory effect and cell death is not fully understood but it has been suggested that Fluoroquinolone-induced growth inhibition and cell death may be mediated through intrinsic apoptotic pathway (Arahna et al., 2000; Bratton et al., 2000; Jurgensmeier et al., 1998; Mancini et al., 1997; Smiley et al., 1991) or through induction of cell cycle arrest by

involvement of different cell cycle molecules (Bratton et al 2000 and Jurgensmeier et al 1998), or by some other way. Since NCI-H460 cell line possesses wild type p53 expression, the possibility of its involvement in the Fluoroquinolone-mediated growth inhibition and apoptosis could not be overruled. Further investigations are currently under progress.

These Fluoroquinolone antibiotics are widely clinically used as antibacterial agents and it has also been reported that none of these Fluoroquinolone are significantly bound to plasma proteins (Sharma et al., 1994). Therefore, penetration of these drugs into fluids and tissues is high and are found to be highly concentrated in the mucosal tissues like lung (Sharma et al., 1994). In a separate study it has also been reported that Fluoroquinolones like Ciprofloxacin that can be administered orally in high dose, can reach concentrations in solid tissues such as lung far above than serum (Herold et al 2002 and Sharma et al 1994). Since, in this study the growth inhibitory effects of Fluoroquinolones were observed at very low concentrations, it appears that certain lung cancers particularly non-small cell lung carcinoma may be responsive to treatment with Fluoroquinolones. Furthermore, use of lower concentration of drug may also minimize the risk of drug toxicity and the development of drug resistance in lung carcinomas. Thus, it is suggested that Fluoroquinolones can be used to control the growth of certain types of non-small cell lung carcinomas.

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