Genotoxic and cytotoxic effects of antibacterial drug, ciprofloxacin, on human lymphocytes *in vitro*

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ABSTRACT

Ciprofloxacin is a bactericidal drug which is being used widely throughout the world for the treatment of various bacterial infection. Cytotoxicity and genotoxicity of Ciprofloxacin on human lymphocytes *in vitro* had been assessed taking various parameters like mitotic index (MI), chromosome aberration (CA), anaphase anomalies, replicative index (RI) and sister chromatid exchange (SCE) as end points. Our results indicate low mitotic index, low replicative index and high frequency of anaphase anomalies on one hand and on other hand, high frequency of chromosome aberration and sister chromatid exchanges (SCEs) in the exposed groups as compared to that of control group. These are the indications of both cytotoxicity and genotoxicity of Ciprofloxacin on human lymphocyte culture *in vitro*. All the parameters obtained from the experimental group were statistically significant when compared to that of control.

Keywords: Ciprofloxacin, genotoxicity, cytotoxicity, chromosome aberration, sister chromatid exchange, anaphase anomalies.

INTRODUCTION

Ciprofloxacin (CFX) is a fluroquinolone antibiotic drug, having broad spectrum antibacterial activity. Reviewing the literature we found increasing number of articles showing adverse effects of the drug. This drug possesses interesting biological properties and potent microcidal activity against urinary tract infection.[1] This synthetic antibiotic compound has been used against gram positive organism.[2] Bactericidal activity of CFX is exhibited in different concentration *in vitro* by binding to bacterial topoisomerase II (DNA gyrase) at their target sites.[3] CFX inhibit DNA replication, repair, transcription, and other cellular functions which cause rapid death of bacteria.[4]

It has been reported that CFX and its quinolone derivatives caused the adverse effect on central nervous system, cardiovascular system, leads to the toxic effect like androtoxicity, reproductive developmental toxicity, carcinogenicity, phototoxicity and genotoxicity.[5,6] CFX caused the biochemical and immunological changes in various cells of the body.[7] It induced apoptosis in activated Jurkat T cell *in vitro*.[8] CFX also produced immunomodulatory effect on monocytes and macrophages.[3,9] *In vitro* genotoxic and cytotoxic effects of CFX had been observed on eukaryotic cells[10] and the lymphocyte growth was inhibited through interference in DNA synthesis.[11] CFX also posses the property to alter *in vitro* function of lymphocytes in the form of genetic abnormality and delay in cell cycle.[10,12] Genotoxicity of CFX has been recorded with sister chromatid exchanges and unscheduled DNA synthesis in human lymphocytes.[13,14]

Now a days CFX is very frequently used with many researchers pointing to the ill effects of CFX, so we designed the study protocol to evaluate the genotoxic and cytotoxic effects of CFX on human lymphocytes in *in vitro* system taking chromosome aberration, mitotic index, anaphase anomalies, sister chromatid exchange (SCE) and replicative index (RI) as end points.

MATERIALS AND METHODS

The study was carried out with six healthy male individuals having mean age 22.66 $\pm$ 0.55 years. Initially 10 individuals were interviewed to rule out any metabolic disorder, infectious disease, smoking habit and mutagenic drug usage in recent period. After screening, six male individuals were selected for the study. Heparinized peripheral blood was taken from these six healthy individuals & lymphocytes cultures were set up with whole blood sample both as control and experimental group (CFX, 40 µg/ml).

LYMPHOCYTES CULTURES:

For each subject, three sets of lymphocyte cultures were set up in duplicate one for control and the other as exposed group. First set of cultures for study of chromosome aberration and mitotic index, second set

Table-1: Cytogenetic Analysis of Peripheral Blood Lymphocyte from Control and CFX Treated Culture Group

No. of Case	Sex	Age	Control				Experimental			
			M.I	C.A	S.C.E	R.I	M.I	C.A	S.C.E	R.I
1	M	23	7.6	00	4.52	2.11	6.85	11	7.54	1.92
2	M	22	7.2	00	5.20	2.10	6.15	10	8.06	1.94
3	M	22	7.6	00	4.76	2.19	6.70	10	7.72	1.89
4	M	23	7.1	02	4.54	2.15	6.20	8	7.26	1.81
5	M	22	8.15	00	4.68	2.11	6.95	12	7.88	1.88
6	M	24	7.5	01	4.82	2.16	6.45	7	8.76	1.91
Mean			7.52 ± 0.34	0.5 ± 0.76	4.75 ± 0.23	2.13 ± 0.0011	6.55 ± 0.307	9.6 ± 1.7	7.87 ± 0.473	1.89 ± 0.04

$P < 0.01$

of culture for anaphase anomalies and third set for sister chromatid exchange and replicative index study. Lymphocytes cultures were set up according to conventional technique i.e. adding 0.5 ml heparinized whole blood to 5 ml of RPMI 1640 medium (Himedia, India) supplemented with 12.0% fetal calf serum (Biological Laboratories, Israel), 2.0% Phytohemagglutinin M (Difco, USA), 100 units/ml penicillin and 100 µg/ml streptomycin. For chromosome aberration and mitotic index study, 40 µg/ml of CFX was added to culture of experimental group whereas no CFX were added to control. For anaphase anomalies, cultures were set up in the same way as that of the culture of chromosome aberration. Third set of cultures for sister chromatid exchange and replicative index study were set up in the same way as mentioned above. But in this culture 5 µg/ml 5-bromo 2'-deoxyuridine (BrdU, Sigma, USA) was added for entire incubation period in both control and CFX treated cultures. All cultures were incubated at 37°C for 72 hrs in complete darkness.

One hour prior to harvesting colchicine (0.25 µg/ml) was added to the two sets of culture. But the cultures for anaphase anomalies study were harvested without introduction of colchicine. Culture were harvested following conventional process and flame dried slides were prepared. The culture set for SCE and RI were followed up to 2 or 3 cell cycles (in both control and CFX treated).

STAINING

Slides for chromosome aberration, mitotic index and anaphase anomalies were stained with Giemsa stain. Slides of sister chromatid exchange and replicative index were stained by fluorescence plus Giemsa staining method.[15] Stained slides were dried and observed under light microscope.

a) Determination of chromosome aberrations

From each blood culture of experimental and control group 5 to 6 slides were used to determine chromosome aberration. 100 metaphases each of expt. and control group were assessed for the chromosome aberration study.

b) Determination of mitotic index:-

To determine mitotic index, 4 to 5 slides from each

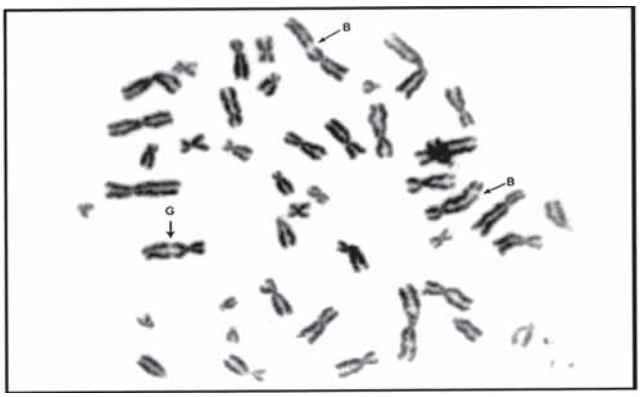

Fig. 1. Metaphase chromosomes showing chromosome break (B) and chromosome gap (G) in CFX treated culture (arrowed), Magnification 1000X

Fig. 2. Sister chromatid exchanges in control culture, five SCEs (arrowed), Magnification 1000X

Table-2: Summary of Cytogenetic Analysis of Blood Lymphocytes from Control group & Experimental Group

	N	Age	M.I	C.A	S.C.E	R.I
Control	6	22.67 ± 0.55	7.52 ± 0.34	0.5 ± 0.76	4.75 ± 0.23	2.13 ± 0.0011
Experimental	6		6.55 ± 0.307	9.6 ± 1.7	7.87 ± 0.473	1.89 ± 0.04

P < 0.01

cultures of control and experimental group were used and a total of 1000 lymphocytes were counted randomly. The mitotic index was calculated as follows.

$$MI = \frac{\text{Number of metaphases}}{\text{Total No. of blast lymphocytes counted}} \times 100$$

c) Determination of anaphase anomalies:-

One hundred anaphase plate from exposed and 100 from control group of each subject were randomly scored. The abnormal anaphases were noted and result expressed in percentage.

d) Determination of SCE and RI :-

For the SCE study, complete second cycle of 50 good spread of metaphases each from control and experimental culture were selected respectively. For replicative index, 100 metaphases of different cell cycle (1st, 2nd and 3rd) were randomly counted. The replicative index was calculated according to the following formula RI= (M1+2M2+3M3)/N, where, M1, M2, M3 indicates metaphases of first, second, third cycle respectively and N is the total number of metaphase scored. .

PHOTOGRAPHY:

The microphotography in this study has been carried out with a Trinocular research microscope (Olympus, model CX 31) by a Digital Camera (Samsung, model SDC - 312) and SLR Camera (Olympus, model SC- 35).

STATISTICAL ANALYSIS:

All the results were expressed as mean ± standard deviation. Student's '*t*' test was used for comparison between control and experimental group. Statistical significance level was set to P<0.01.

RESULTS

The results of Cytogenetic analysis of CFX treated *in vitro* lymphocytes cultures were presented in Table-1 and 2. This data showed genotoxic and cytotoxic effect of CFX on lymphocytes cultures of all six individuals. In control, range of mitotic index was 7.1 to 8.15 and in experimental culture it was 6.15 to 6.96. Mean and S.D. of MI in control and expt. was (7.52 ± 0.34) and (6.55 ± 0.31) per 100 cells respectively. The chromosome aberrations range in control was 1.0-2.0% and expt. group 7.0-12.0%. The chromosome aberration analysis in control group revealed (0.5 ± 0.76) per 100 cells while treated culture exhibited (9.6 ± 1.7). In all experimental culture many different types of abnormalities ie chromosome breaks, chromosome gaps, chromatid breaks and chromatid gaps were recorded. Chromosomal aberrations were found to occur in significantly higher frequencies in experimental group as compared to that of control. The range of sister chromatid exchanges (Fig-2) in control group was 4.52 to 5.20 and experimental group was 7.26 to 8.76 per cell. The frequency of sister chromatid exchanges in control group was (4.75 ± 0.23) while increase in frequency occurs up to (7.87 ± 0.47) per cell in experimental group. The range of replicative index in control group was 2.10 to 2.19 and experimental group was 1.81 to 1.91. The replicative index in control

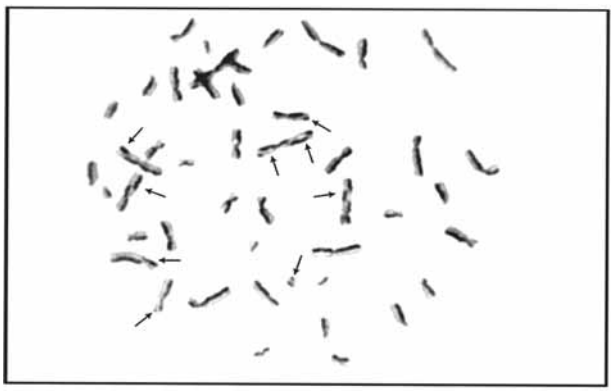

Fig. 3. Sister chromatid exchanges in CFX treated culture, nine SCEs (arrowed), Magnification 1000X

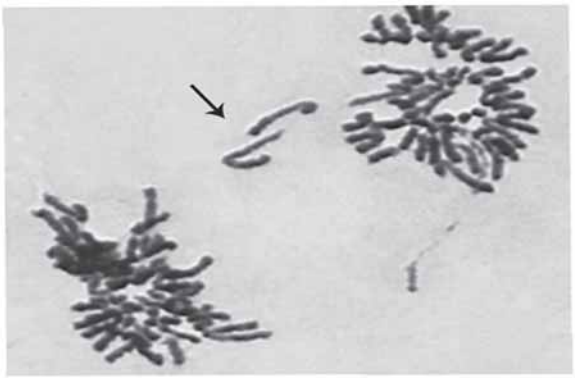

Fig. 4. Photograph showing lagging chromosome at anaphase in exposed culture (arrowed), Magnification 1000X

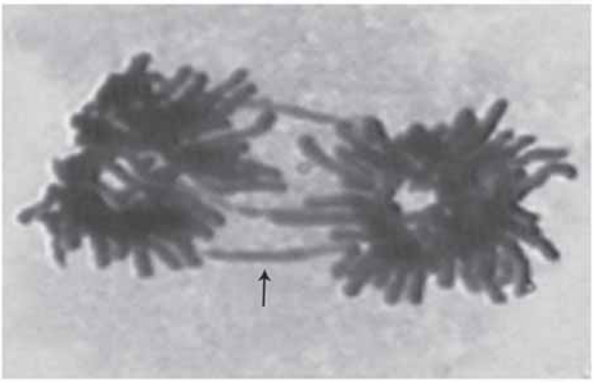

Fig. 5. Photograph showing anaphase bridge in CFX exposed culture arrowed), Magnification 1000X

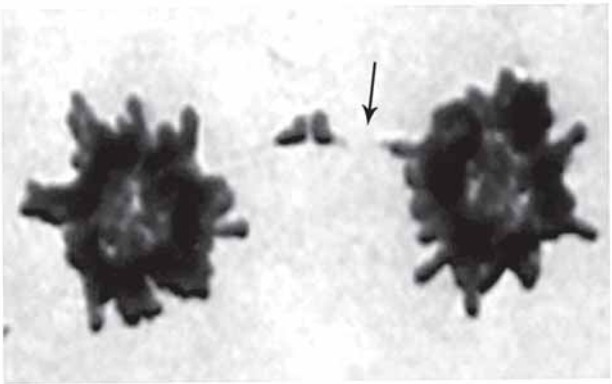

Fig. 6. Photograph showing Chromosome break followed by lagging chromosome fragments at anaphase from exposed culture (arrowed), Magnification 1000X

group was (2.13 ± 0.0011) which was decreased in CFX treated group to (1.89 ± 0.041). In the study of anaphase abnormalities, 4.0-6.0% anomalies were observed in the form of anaphase bridge, lagging chromosome, chromosome break in experimental culture.

We observed cytotoxic and genotoxic effects of CFX on human lymphocytes culture *in vitro* by using above parameters. Significantly decreased frequencies of mitotic index and replicative index (P<0.01) were recorded. Statistically increased chromosome aberration and sister chromatid exchange frequencies were obtained in CFX treated culture group as compared to the control (P<0.01).

DISCUSSION

Ciprofloxacin is one of the Quinolone groups of antibacterial drug which is currently available for clinical use all over the world. CFX acts on bacterial DNA by inhibiting the activity of topoisomerase II (DNA gyrase).[3] In both prokaryotes and eukaryotes, topoisomerase II enzyme has a multifunctional activity in all cellular process involving DNA replication, transcription and repair[16] along with chromosome condensation and chromatid separation in eukaryotic cells.[4] Previous studies reported that CFX caused chromosome aberration and sister chromatid exchange in human lymphocytes culture in *vitro*.[10,13,17]

In the present study the genotoxic and cytotoxic effect of CFX on human lymphocytes were evaluated in exposed culture compared with that of control *in vitro*. Different types of aberrations such as chromosome break or gap (Fig1) and chromatid break or gaps(Fig-3) were recorded. Higher percentage of chromosome aberration (Fig. 1) and significant increase in sister chromatid exchanges (Fig. 3) were noted in exposed culture group when compared with that of control (Fig. 2). Decreasing mitotic index and replicative index also observed in CFX exposed culture when compared to control (Table-1).

CFX induce the chromosome aberration in human lymphocyte cells culture *in vitro*, by irreversible breakage in DNA and enhancing eukaryotic topoisomerase II mediated DNA cleavage *in vitrowere also reported* .[10,18,19] CFX directly binds to gyrase - DNA complex, stabilizing it and disturb the resealing activity of DNA gyrase and preventing enzyme turn over. Which is the possible mechanism of action.[3,4] It forms the complex known as "cleavable complex"[20] which cause breakage in DNA or chromosome (Fig. 1 and 6). It may occur at any stage of mitosis.[13]

Anaphase analysis of the CFX exposed culture showed lagging chromosomes (Fig. 4 and 6). DNA topoisomerase II activity is necessary for mammalian sister chromatid separation.[2] CFX possibly inhibit DNA topoisomerase activities and there by interferes with anaphase separation and produce abnormalities of segregation that leads to lagging chromosome[21] or micronucleus formation.[22] This episode may cause aneuploidy of the cell.[23] In another condition, anaphase chromosome bridge (Fig. 5) formation was observed. The broken ends of the chromosome might have fused and formed this bridge like configuration as suggested earlier.[24] This may result in misseggregation or loss of genes which culminate in to cell death.

Decreased mitotic index and replicative index of CFX induced lymphocytes culture showed the cytotoxic effect of drug. CFX inhibit DNA gyrase and Topoisomerase II, which is important for resolving the superhelical interwinded structure of DNA during replication.[25] It also interfered in segregation of chromosome at anaphase in mitosis consequently resulting in the delay of cell cycle[26] and prolongs metaphase stage.[22]

Another interesting finding we noted was fuzzy morphology of 8.0-10.0% metaphase chromosome in CFX treated culture. It was suggested that CFX might have interacted with DNA associated Scaffold protein like acidic nonhistone chromosomal protein[27] from their

binding sites and induce potential DNA lesion or fuzzy metaphase.[10] Around 8.0% of metaphases showing chromosome decondensation. This may be due to the action of CFX on DNA topoisomerase II which is necessary for proper condensation of chromosome.[28]

It was evident from our result that CFX induce cytotoxic and genotoxic effects in human lymphocytes *in vitro*. We are to consider the genotoxicity and cytotoxicity of CFX on human cells *vis a vis* the bactericidal activities of the drug. Result indicates a warning signal to injudicious and indiscriminate use of the drug.

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