**EXPERIMENTAL / LABORATORY STUDIES**

# Genotoxic Evaluation of the Antibacterial Drug, Ciprofloxacin, in Cultured Lymphocytes of Patients with Urinary Tract Infection

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**Abstract:** Ciprofloxacin is a quinolone carboxylic acid derivative and is commonly used in medicine. The genotoxicity of ciprofloxacin was evaluated in cultured human peripheral blood lymphocytes in patients with urinary tract infection. Sister chromatid exchange (SCE), mitotic index (MI) and replicative index (RI) were measured before and after ciprofloxacin therapy.

Our results showed that SCE frequency significantly increased after ciprofloxacin therapy ($P < 0.001$), but MI and RI decreased ($P < 0.001$). The results of this study suggest that clinical therapy using ciprofloxacin may have a moderate genotoxic potential.

**Key Words:** Ciprofloxacin, sister chromatid exchange, mitotic index, replicative index

## Introduction

Ciprofloxacin (CFX) is a fluoroquinolone and is widely used in medicine. CFX is highly active in vitro against a broad spectrum of Gram-negative and Gram-positive organisms (1). The fluoroquinolones exhibit concentration-dependent bactericidal activity and exert their activity by binding to bacterial topoisomerases II (DNA gyrase) and IV. By binding to these bacterial target sites, quinolones interfere with DNA replication, repair, and transcription as well as with other cellular functions, rapidly leading to bacterial death (2). Therefore, these drugs are widely used in clinical practice.

It has been reported that quinolones have some toxic effects on the central nervous, cardiovascular and gastrointestinal systems, and that they also lead to ondrotoxicity, reproductive and developmental toxicity, genotoxicity, carcinogenicity and phototoxicity (3,4).

In vitro genotoxicity of CFX has been demonstrated with sister chromatid exchange (SCE) and unscheduled DNA synthesis (5). In vivo genotoxicity of CFX has been demonstrated with the micronucleus test (6) and chromosomal aberrations in lymphocytes of humans (7), mice (8) and rats (9). However, none of the patients treated with CFX for long periods have shown signs of chromosomal aberrations (10-11).

SCE consists of reciprocal exchanges between sister chromatids. The mechanism of SCE involves DNA damage and repair mechanism defects may play an important part in this (12). SCE frequency is, therefore, a more sensitive marker of mutagenesis than are chromosomal abnormalities, although they occur after exposure to many genotoxic agents (13) or various diseases (14) and are believed to indicate DNA damage. The SCE phenomenon is widely used as a reliable indicator of chromosome or DNA instability.

To our knowledge, there have been no published studies investigating SCE frequencies in patients treated with CFX. The aim of the present study was to investigate any possible effects of CFX on SCE, mitotic index (MI), and replicative index (RI) in the peripheral lymphocytes of patients.

## Materials and Methods

### Patients

The study was carried out in 22 patients with urinary tract infection (UTI) and in 17 healthy controls. Only *Escherichia coli* positive samples were included in the study.

Patients with UTI were included in the study by interview and were chosen from among individuals with no metabolic or infectious disease, non-smokers, with no history of long-term mutagenic drug use and who were diagnosed with UTI for the first time. Heparinized peripheral blood was taken from the patients before treatment and 1 week after treatment (CFX, 500 mg/day).

Patients were treated with CFX (10 males, 12 females; mean age 30.05 ± 1.33 years). The control group was composed of individuals with no metabolic disease and who were non-smokers (8 males, 9 females; mean age 29.59 ± 1.57 years).

### Lymphocyte cultures

Lymphocyte cultures were set up by adding 0.5 ml of heparinised whole blood to RPMI-1640 chromosome medium supplemented with 15% heat-inactivated foetal calf serum, 100 IU/ml streptomycin, 100 IU/ml penicillin and 1% L-glutamine. Lymphocytes were stimulated to divide by 1% phytohaemaglutinin. For SCE demonstration, the cultures were incubated at 37 oC for 72 h, and 5-bromo 2$^l$-deoxyuridine (BrdU) at 8 mg/ml was added at the initiation of cultures. All cultures were maintained in darkness. Next, 0.1 mg/ml of colcemide was added 3 h prior to harvesting to arrest the cells at metaphase. Cells were harvested and treated for 30 min with hypotonic solution (0.075 M KCL) and fixed in a 1:3 mixture of acetic acid/methanol (v/v). Bromodeoxyuridine-incorporated metaphase chromosomes were stained by fluorescence plus Giemsa technique as described by Perry and Evans (15). Two to three slides were stained with Giemsa solution (2.5%) for the demonstration of the MI.

### SCE, RI and MI analysis

In the SCE study, by selecting 30 satisfactory metaphases, the results of SCE were recorded on the evaluation table. One hundred metaphases per patient were also scored to determine the proportion of cells that undergo 1, 2 and 3 divisions. The replicative index (RI) was calculated according to the formula RI = (M1 + 2M2 + 3M3) / N, where M1, M2 and M3 indicate those metaphases corresponding to the first, second and third divisions and N the total number of metaphases scored (16). For the analysis of the MI, the number of mitotic cells per at least 500 cells was used.

The Mann-Whitney U test was used between the control and pretreatment patient groups. The Wilcoxon signed ranks test was applied for the comparison of pretreatment and post-treatment values obtained from the patient group.

## Results

Tables 1-3 show the mean (SE) SCE frequencies, MI and RI of controls and patients before and after CFX therapy. Mean SCE frequencies, and MI and RI between control and pretreatment patient groups were not statistically significant (P < 0.05).

Table 2 shows the results of the genotoxic evaluation of CFX in lymphocyte cultures using SCE, MI, and RI. We observed an effect of CFX on the MI and RI of lymphocyte cultures. A significant decrease in the frequency of cellular division was detected (P < 0.001).

Statistically increased SCE frequencies were obtained in the post-treatment group as compared to the pretreatment and control groups (P < 0.001). Table 3 presents all the features of the groups.

## Discussion

CFX is one of the best known drugs for the treatment of many bacterial infections. In the literature, the antibacterial feature of the drug has been attributed to DNA binding, resulting in a given inhibition of bacterial DNA topoisomerases (2).

In our study, the effect of the drug on lymphocyte cultures was examined. Genotoxic effects of CFX were observed in the patient group. The genotoxic effect was particularly seen on increased SCE frequencies and decreased MI and RI. As can be seen in Table 3, the distribution of values for SCE, MI and RI in the control group was statistically different from that for the patient group. Although there were no large differences between the control and patient groups who did not receive CFX regarding SCE, MI and RI, the genotoxic effect of CFX in

Table 1. Cytogenetic analysis of peripheral blood lymphocytes from the control group.

| | Age (years) | Sex | MI | PRI | SCE/Cell |
|---|---|---|---|---|---|
| 1 | 28 | M | 5.5 | 1.76 | 6.8 |
| 2 | 31 | M | 6.0 | 1.70 | 7.1 |
| 3 | 44 | F | 5.1 | 1.72 | 7.4 |
| 4 | 17 | F | 7.6 | 1.79 | 8.5 |
| 5 | 25 | M | 6.5 | 1.82 | 7.1 |
| 6 | 29 | F | 8.4 | 1.68 | 7.2 |
| 7 | 31 | M | 6.8 | 1.72 | 7.3 |
| 8 | 31 | M | 7.3 | 1.86 | 6.7 |
| 9 | 40 | F | 5.7 | 1.79 | 6.5 |
| 10 | 27 | F | 10.4 | 1.71 | 8.1 |
| 11 | 31 | M | 8.7 | 1.85 | 6.8 |
| 12 | 34 | F | 7.4 | 1.78 | 6.4 |
| 13 | 34 | M | 8.3 | 1.66 | 6.9 |
| 14 | 21 | F | 5.6 | 1.72 | 6.6 |
| 15 | 29 | M | 49 | 1.40 | 6.8 |
| 16 | 23 | F | 6.7 | 1.66 | 6.9 |
| 17 | 28 | F | 8.3 | 1.71 | 7.1 |
| Mean ± SE | | | 7.01 ± 0.36 | 1.73 ± 0.02 | 7.07 ± 0.13 |

Table 2. Cytogenetic analysis of blood lymphocytes from patient group at pretreatment and post-treatment periods.

| | Age (years) | Sex | Before treatment | | | After treatment | | |
|---|---|---|---|---|---|---|---|---|
| | | | MI | PRI | SCE/Cell | MI | PRI | SCE/Cell |
| 1 | 27 | M | 6.8 | 1.65 | 7.2 | 5.4 | 1.48 | 8.4 |
| 2 | 35 | F | 6.0 | 1.56 | 7.1 | 5.3 | 1.44 | 9.3 |
| 3 | 33 | F | 5.9 | 1.80 | 8.8 | 6.1 | 1.76 | 10.1 |
| 4 | 29 | F | 7.1 | 1.72 | 6.4 | 5.8 | 1.62 | 8.9 |
| 5 | 32 | M | 7.0 | 1.78 | 6.9 | 4.9 | 1.86 | 8.5 |
| 6 | 36 | F | 7.4 | 1.66 | 7.1 | 4.7 | 1.52 | 9.4 |
| 7 | 25 | F | 8.0 | 1.68 | 7.2 | 5.6 | 1.34 | 9.6 |
| 8 | 38 | M | 8.5 | 1.80 | 6.8 | 7.1 | 1.46 | 8.2 |
| 9 | 25 | M | 7.3 | 1.51 | 6.4 | 6.8 | 1.38 | 7.8 |
| 10 | 24 | F | 6.8 | 1.68 | 6.3 | 5.3 | 1.48 | 8.6 |
| 11 | 27 | F | 7.4 | 1.72 | 6.4 | 6.1 | 1.56 | 8.2 |
| 12 | 17 | M | 7.2 | 1.44 | 7.1 | 6.4 | 1.24 | 9.3 |
| 13 | 25 | F | 4.1 | 1.81 | 6.8 | 4.5 | 1.78 | 9.1 |
| 14 | 27 | M | 6.8 | 1.78 | 5.4 | 5.2 | 1.68 | 7.8 |
| 15 | 41 | F | 7.4 | 1.66 | 6.3 | 6.2 | 1.54 | 6.9 |
| 16 | 38 | F | 6.8 | 1.85 | 7.2 | 6.1 | 1.72 | 8.6 |
| 17 | 35 | M | 7.3 | 1.71 | 6.9 | 5.9 | 1.66 | 8.3 |
| 18 | 29 | M | 6.5 | 1.72 | 6.8 | 5.5 | 1.42 | 8.4 |
| 19 | 33 | F | 7.1 | 1.64 | 7.2 | 5.9 | 1.36 | 8.6 |
| 20 | 31 | F | 6.8 | 1.68 | 5.8 | 5.6 | 1.45 | 8.1 |
| 21 | 19 | M | 6.7 | 1.71 | 6.9 | 6.1 | 1.35 | 7.8 |
| 22 | 35 | M | 7.2 | 1.74 | 7.1 | 4.8 | 1.54 | 7.6 |
| Mean ± SE | | | 6.91 ± 0.18 | 1.70 ± 0.02 | 6.82 ± 0.14 | 5.69 ± 0.14* | 1.52 ± 0.02* | 8.52 ± 0.16* |

* $P < 0.001$

Table 3. Summary of cytogenetic analysis of blood lymphocytes from the control group and patients with UTI.

|  | n | Age | MI | RI | SCE |
|---|---|---|---|---|---|
| Control | 22 | 29.59 ± 1.57 | 7.01 ± 0.36 | 1.73 ± 0.02 | 7.07 ± 0.13 |
| Before Treatment |  |  | 6.91 ± 0.18 | 1.70 ± 0.02 | 6.82 ± 0.14 |
|  | 17 | 30.05 ± 1.33 |  |  |  |
| After Treatment |  |  | 5.69 ± 0.14* | 1.52 ± 0.02* | 8.52 ± 0.16* |

* $P < 0.001$: before treatment vs after treatment

the patient group can clearly be seen to cause increased SCE frequencies and decreased MI and RI (Tables 2 and 3).

DNA gyrase or topoisomerase II, which is essential to life, plays a role in establishing the structure of transcriptionally active chromatin, reducing torsional strain and resolving intertwined strands during DNA replication, condensation of chromosomes during mitosis, and chromosome disjunction at anaphase (2). Inhibition of topoisomerase II prolongs metaphase and interferes with the separation of sister chromatids at anaphase, but does not prevent cells from undergoing a cleavage that results in chromosome abnormalities and non-disjunction (17). Fluoroquinolones have exhibited varying degrees of cross-reactivity with mammalian topoisomerases II and other replication enzymes. These compounds may thus have the potential to induce DNA lesions by interacting with the DNA associated protein with regard to quinolone interaction, topoisomerase II, because of its structural and functional similarity to bacterial gyrase. CFX appears to exert its genotoxic action through binding to the gyrase-DNA complex, stabilising it and preventing enzyme turnover. This complex is termed a "cleavable complex" (18). The formation of this complex results in a double-strand break in the helix with both free ends of the helix attached to the enzyme by way of phosphotyrosine linkages. It is likely that cleavable complex stabilisation plays a key role in CFX genotoxic activity and this may occur at multiple stages in the cell cycle, including mitosis,

but there may be other mechanisms of quinolone involvement in topoisomerase II-mediated DNA damage (19). Genotoxicity was also observed with some pharmaceuticals as other topoisomerase interactive (20) and parasitic agents (21) revealed high increases in chromosomal aberrations of therapeutic concentrations (6).

We think that our results, together with other published data, indicate that the antimicrobial drug CFX is able to induce both cytotoxic and moderate genotoxic effects in cultured human lymphocytes; these cytogenetic effects being compatible with the role of inhibition of topoisomerase in the DNA metabolism.

Further in vitro and in vivo studies are needed before definitive conclusions about the mutagenic potential of CFX and other quinolones can be drawn. This potential is one reason why CFX and other quinolones are not to be used in children, adolescents or in pregnant and lactating women.

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