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EVALUATION OF GENOTOXIC EFFECTS OF LEVOFLOXACIN IN HUMAN PERIPHERAL LYMPHOCYTES

KEYWORDS: Genotoxicity, Levofloxacin, Chromosomal aberrations, Sister chromatid exchanges, Micronucleus

Ahmet KAYRALDIZ
University of Kahramanmaraş Sütçü İmam, Science and Letters Faculty, Depatment of Biology, Kahramanmaraş, Turkey

İbrahim Halil KENGER
University of Kahramanmaraş Sütçü İmam, Science and Letters Faculty, Depatment of Biology, Kahramanmaraş, Turkey

Lale DÖNBAK
University of Kahramanmaraş Sütçü İmam, Science and Letters Faculty, Depatment of Biology, Kahramanmaraş, Turkey

Zeliha YILDIRIM
University of Gaziantep, İslahiye Vocational School, Department of Veterinary, İslahiye/Gaziantep, Turkey

ABSTRACT

Background and Objectives: Levofloxacin, is a third generation quinolon antibiotic used in the the treatment of upper respiratory tract, urinary tract, skin and soft tissue, bone-joint, eye and ear infections. In this study, possible genotoxic effects of levofloxacin were analyzed by using sister chromatid exchanges (SCE), chromosome aberrations (CA) and micronucleus (MN) tests in human peripheral lymphocytes.

Methods: Peripheral blood samples taken from two healthy women and two men were treated with four different concentrations of levofloxacin (12,5, 25, 50 and 100 µg /ml) for 24 and 48 hours and SCE, CA, and MN levels of treated cells were examined. In order to analyze the cytotoxic effect, mitotic index, proliferation index and nuclear division index in the treated cells were also determined. The obtained data was compared with the control by using SPSS (17.0) software programme.

Results: Treatment with the different doses of levofloxacin for 24 and 48 h did not effect the SCE frequency, but highest levofloxacin dose (100 µg/ml) caused a significant increase in the CA level. Also, treatment of 25, 50, and 100 µg/ml levofloxacin significantly increased the MN level as compared the control group. There was no significant differences between the treated cells and control according to the proliferation index, mitotic index, and nuclear division index. In conclusion, levofloxacin has genotoxic but not cytotoxic effect in human peripheral lymhocytes.

INTRODUCTION

Fluoroquinolones are one of the most commonly prescribed classes of antibacterials in the world and extensively used for treatment of the infections caused by Gram-positive and Gram-negative bacteria, mycobacteria, and parasites in humans. Fluoroquinolones haltes the bacterial DNA replication and cell divisionn by inhibiting DNA gyrase (bacterial topoisomerase II) or inhibite the topoisomerase IV by induction of SOS pathway for DNA repair and also have slight inhibitory effect on eukaryotic topoisomerase II[1].

Levofloxacin is a synthetic broad-spectrum antibiotic of the fluoroquinolone drug class that is widely used in the treatment of mild to moderate respiratory and urinary tract infections[2,3]. However, as with most drugs, fluoroquinolones can lead to a wide extent of side effects ranging from mild to severe. Besides to common adverse effects such as nausea, headache, diarrhea, insomnia, fluoroquinolone usage including levofloxacin have been associated with anaphylaxis, central nervous system effects including seizures and psychiatric effects, prolongation of the QT interval, blood glucose disturbances, hepatotoxicity, and photosensivity[4-7]. Morever, several researchers indicated carcinogenic, mutagenic, or genotoxic effects of fluororoquinolones, such as ciprofloxacin, gemifloxacin, and norfloxacin, in different test systems along with other adverse recations[8-11].

Concerning the levofloxacin, it has been reported to not have mutagenic effect on *Salmonella typhimurium, Esherichia coli* (Ames test) and Chinese hamster ovary (CHO) cells (HGPRT test) and not induce micronucleus (MN) and sister chromatid exchange (SCE) frequencies in Mouse bone marrow, whereas, levofloxacin showed genotoxic effects in Chinese hamster lung (CHL) cell line by *in vitro* SCE and chromosomal aberations (CA) tests[12], in another study levofloxacin increased the frequency of cells containing structural aberrationsin in CHL cells[13].

Due to lack of knowledge about genotoxicity of levofloxacin in cultured human lymphocytes, in this study, possible genotoxic effects of levofloxacin were investigated in human lymphocytes by using *in vitro* SCE, CA, and MN tests, Beside, proliferation index (PI), mitotic index (MI), and nuclear division index (NDI) values were investigated to determine the possible cytotoxic effect of levofloxacin.

MATERIAL AND METHODS

In this study, Tavanic tablet (500 mg, Sanofi Aventis) whose active substance is levofloxacin has been used as test substance. Levofloxacin is an synthetic antibiotic and chemically is optical L-isomer of the medication ofloxacin Chemical formula of levofloxacin is $C_{18}H_{20}FN_3O_4$ (Fig. 1)..

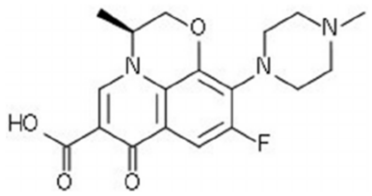

Fig. 1. Chemical structure of levofloxacin.

Levofloxacin concentrations that would be used in the study were determined based on daily dosage of an adult (2 tablets, 1024.92 mg levofloxacin). For preparing the 50 µg/ml stock solution of levofloxacin, which have levofloxacin equivalent to an adults daily dosage, a Tavanic tablet was crushed and converted into powder and then 0.04972 gr powder was solved in 50 ml pure water. Other dosages were also arranged in the form of increasing and reducing folds of stock solution and 12,5, 25, and 100 µg/ml of levofloxacin dosages were prepared. So, four different levofloxacin dosages, 12,5, 25, 50, and 100 µg/ml, were used in the experiments.

Cell Cultures

For cell cultures, 5 ml of intravenose blood sample was taken from healthy and non-smoking two women and two men between 22-25

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ages and was heparinised in the ratio of 1/10. For SCE and CA tests, 6 drop of blood samples were added to tubes containing chromosome medium (Gibco, 12552-013). Then, 50 µl BrdUrd solution was added to the tubes and cell cultures were incubated at 37 °C for 72 hours. Lymphocytes were treated with 12.5, 25, 50, and 100 µg/ml levofloxacin for 24 and 48 hours in cell culture. At the 70 th hour of the incubation, 25 µl colchicine (Sigma, C-3915) was added to tubes for inhibiting microtubule polymerization. Also, mitomycin C (Sigma, M-0503) was used as positive control for both 24 and 48 hours treatments. Preparates for SCE and CA analysis were prepared according to Perry and Thomson[14] and Evans[15].

For MN test; 6 drops of heparinised blood samples were added to chromosome medium and tubes were incubated at 37 °C for 72 hours. Lymphocytes were treated with levofloxacin dosages for 24 and 48 hours. At the 44th hour of incubation 16 µl cytochalasin-B (Sigma, C-6762) was added to tubes to cytokinesis blocking in cells. Preparates for MN test were prepared by modifying the method developed by Rothfuss et al[16].

Microscobic Examinations

In microscobic examinations, SCE frequency was determined in 25-well spread metaphase of second mitotic division for each person and concentrations and sister chromatid exchange per cell (SCE/Cell) were calculated. 100 well-spreated metaphases was investigated and chromosomal aberrations were recorded for CA analysis. In these examined cells, the percentage of cells containing abnormality (AC) was also recorded. For MN analyzis, frequencies of MN and binucleated cell containing micronucleus (BNMN) were determined in 1000 binucleated cells. In order to determine the cytotoxic effect of levofloxacin, proliferation index (PI), mitotic index (MI), and nuclear divison index (NDI) were also determined for each sample.

Statistical Analyzis

The data obtained from microscobic examinations were compared with control group by using t-test in SPSS (17.0) packet programme. 5 % level of significance ($p \leq 0.05$) was considered as important.

RESULTS

Mean values of cytogenetic markers determined in human peripheral lymphocytes treated with 12,5, 25, 50, and 100 µg/ml levofloxacin concentrations were given in Table 1,2, and 3. When the data obtained from cells treated with levofloxacin for 24 and 48 h were compared to control group, no important difference was determined in the SCE/Cell frequency for all levofloxacin concentrations ($p>0.05$) (Table 1). With respect to PI, there was also no statistically significant differences between the treated cells and control group ($p>0.05$).

Table 1. Mean values of sister chromatid exchanges per cell (SCE/Cell) and Proliferation index (PI) in human peripheral lymphocytes treated with different dosages of levofloxacin for 24 and 48 h.

Test substance	Treatment		SCE/Cell±SE	PI±SE
	Duration (h)	Dosage (µg/ml)		
C[a]	24	0.00	1.97±0.21	2.52±0.07
MMC[b]		0.10	31.95±1.29	1.36±0.18
Levoflo.		12.50	2.66±0.29	2.45±0.05
		25.00	2.72±0.26	2.53±0.02
		50.00	3.00±0.39	2.51±0.06
		100.00	2.57±0.20	2.55±0.07
C	48	0.00	1.97±0.21	2.52±0.07
MMC		0.10	59.67±0.98	1.36±0.12
Levoflo.		12.50	3.00±0.43	2.61±0.07
		25.00	2.39±0.40	2.60±0.03
		50.00	2.20±0.35	2.52±0.05
		100.00	2.65±0.29	2.53±0.05

a : Control
b : Positive control, Mitomycin C
* $p<0.05$; ** $p<0.01$

As seen in Table 2, CA/Cell and AC frequencies were determined as 0.04±0.01 and 4.00±0.70 in control group, respectively. When data were compared with that of treated cells, according to CA and AC percentage, it was determined that CA and AC rate in cells exposed to 100 µg/ml levofloxacin for 24 and 48 h were significantly higher as to control group ($p<0.01$). In examined cells from CA preparates, structural CA was observed as chromatid and chromosome breaks, fragment, dicentric chromosome and endoreduplication. Polyploidy was also encountered as numerical abnormality (Table 2). On the other hand, no significant difference in the MI values was determined between the treated cells and the control group ($p>0.05$).

Table 2. Mean values of Chromosomal aberrations per cell (CA/Cell), Abnormal cell (AC), Mitotic index (MI) and the number of observed chromosomal abnormalities in human peripheral lymphocytes treated with levofloxacin.

Test Substance	Treatment		CA/ Cell±SE	Chromosome Abnormalities[a]							AC±SE (%)	MI±SE
	Dura. (h)	Doses (µg/ml)		B'	B"	F	SU	DS	ER	P		
C[b]	24	0.0	0.04±0.01	8	3	0	0	1	0	4	4.00±00.70	0.05±0.00
MMC[c]		0.1	0.24±0.01	28	19	6	26	5	8	4	24.25±0.85	0.02±0.00
Levoflo.		12.50	0.04±0.00	10	3	0	0	2	0	4	4.75±0.75	0.05±0.00
		25.00	0.06±0.01	12	7	1	0	1	0	5	6.50±1.19	0.06±0.00
		50.00	0.05±0.01	10	5	2	0	2	0	3	5.50±1.19	0.05±0.00
		100.00	0.08±0.00**	15	7	2	0	2	2	4	7.50±0.64**	0.05±0.01
C	48	0.0	0.04±0.00	8	3	0	0	1	0	4	4.00±0.70	0.05±0.00
MMC		0.1	0.37±0.02	42	35	16	17	10	25	3	36.25±1.88	0.02±0.00
Levoflo.		12.50	0.05±0.01	10	4	1	0	2	0	3	5.00±1.77	0.06±0.00
		25.00	0.05±0.01	9	5	1	0	1	0	4	5.00±1.00	0.05± 0.00
		50.00	0.07±0.01	12	5	3	0	3	0	5	7.00±1.58	0.05±0.00
		100.00	0.08±0.00**	17	4	3	0	3	0	5	7.50±0.50**	0.06±0.00

[a]B', Chromatid Brekage; B", Chromosome Brekage; F, Fragment; SU, Combination of sister Chromatids, DS, Dicentric Chromosome; ER, Endoreduplication; P, Poliploidy; [b] : Control [c] : positive control, Mitomycin C
* $p<0.05$; ** $p<0.01$

As seen in Table 3, except for the lowest dose (12,50 µg/ml), an important increase was observed in MN and BNMN frequency in cells treated with 25, 50 ve 100 µg/ml levofloxacin for 24 and 48 h compared to control group ($p<0.01$). When obtained data were compared in terms of NBI, no important difference was observed in NBI value at all levofloxacin doses and durations compared to control group($p>0,05$).

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Table 3. Mean values of Micronucleus (MN), Micronucleated binucleat cell (BNMN), and Nucleer division index (NBI) in human peripheral lymphocytes treated with levofloxacin.

Test substance	Treatment		MN±SE (‰)	BNMN ±SE (‰)	NBI±SE
	Dura. (h)	Dosage (µg/ml)			
C[a]	24	0.00	5.50±0.95	4.75±0.85	2.13±0.05
MMC[b]		0.10	23.25±1.03	20.00±0.91	1.08±0.02
Levoflo.		12.50	9.00±2.04	8.50±1.84	2.17±0.11
		25.00	12.50±1.32**	10.75±1.88*	1.99±0.04
		50.00	10.75±0.95**	9.75±0.62**	1.97±0.04
		100.00	11.50±0.86**	10.75±1.25**	1.83±0.05
C	48	0.00	5.50±0.95	4.75±0.85	2.13±0.05
MMC		0.10	52.25±2.01	48.75±1.25	1.08±0.02
Levoflo.		12.50	10.50±2.39	9.75±2.28	2.14±0.09
		25.00	13.75±0.75**	12.50±0.086**	2.19±0.03
		50.00	10.75±1.03**	9.00±0.81**	2.17±0.05
		100.00	12.75±1.65**	10.50±0.64**	2.04±0.04

a : Control
b : Pozitive control, Mitomycin C
* p<0.05; ** p<0.01

DISCUSSION

Due to importance of determining the genotoxic and cytotoxic effects of the antibiotics as well as other various side effects, several studies has been conducted on the possible genotoxic potentials of fluoroquinolones including levofloxacin by using different *in vivo* and *in vitro* test systems[8-11]. Itoh et al[13], examined the photochemical clastogenic potential of 12 antibacterial agents from quinolone group (sparfloxacin, clinafloxacin, gemifloxacin, lomefloxacin, sitafloxacin, grepafloxacin, fleroxacin, enoxacin, levofloxacin, moxifloxacin, trovafloxacin, and DK-507k) in cultured CHL cells by *in vitro* chromosomal aberration test. Following the light radiation, it was observed that antibacterial agents (except DK-507k) have caused to an increse in the frequency of cells containing structural aberrations. Researchers reported that photochemical and non-photochemical clastogenic potentials of quionole antibacterial agents have decreased by the displacement of methoxy group at the C-8 position of quinole core.

Reus et al[17], have found that sporloxacin and lomefloxacin significiantly increased MN frequency in the skin of mouse by *in vivo* photomicronucleus test but the increase observed in treated cells with ciprofloxacin and levofloxacin was not at significant level. Itoh et al[18], reported that levofloxacin (300 and 600 mg/kg) did not increase the mutant frequency in the bone marrow, liver, testis and sperm cells in lacZtransgenic mice (Muta™Mouse) and under these experimetal conditions levofloxacin was not mutagenic on lacZtransgenic mice.

Shimada[12], investigated the mutagenic effect of levofloxacin by using Ames test, HGPRT mutation test, SCE test in CHL cells, MN, CA, and SCE test in mouse bone marrow, UDS test in rat primary hepatocytes and the dominant lethality test in BDF1 mice. Levofloxacin, induced CA and SCE frequency in a dose dependent manner in CHL cells, whereas no mutagenic effect was observed in other tests.

Zhu et al.[19], have investigated the genotoxic effect of levoflaxacin n-oxid isolated from levoflaxacin by CA test in CHL cells in the presence and absence of S9 mix. Although the test substance caused a significant increase in the number of metaphases with structural aberration, no significant elevation has been observed in the frequency of structural aberrations. Zhu et al.[20] found that decarboxylated levofloxacin isolated from levofloxacin did not show mutagenic activity in the Ames test, but significantly increased the number of cells containing structural aberration in CHL cells rather than structural aberrations.

Tan et al.[21] found that levofloxacin significantly reduced cell viability and hyaluronan level, while increasing apopitosis and active caspase-3 levels in rabbit fibroblast-like synoviocyte cells (FLS) and indicated that levofloxacin have cytotoxic effect on FLS cells. Deng et al.[22] observed that levofloxacin increased apoptosis in rabbit anterior cruciate ligament cells (ACL) treated with levofloxacin but reduced the amount of extracellular matrix and they reported that levofloxacin have cytotoxic effect on these cells.

Due to lack of knowledge about genotoxicity of levofloxacin in cultured human lymphocytes, in this study, possible genotoxic effects of levofloxacin were investigated by using *in vitro* SCE, CA, and MN tests. In this study, treament with different doses of levofloxacin (12.50, 25, 50, and 100 µg/ml) for 24 and 48 hours did not effect SCE frequency of treated cells in contrast to the study of Shimada[12] in which levofloxacin increased SCE level dose dependently. On the other hand, there was a significant increase in the CA and AC levels of cells treated with 100 µg/ml levofloxacin (higgest dose) as compared to control group. CA results of this study are consistent with the findings of Shimada[12] from the CHL cells. Similarly, 25, 50, and 100 µg/ml levofloxacin resulted in a significant increase in MN and BNMN frequency of treated cells in contrast to the studies of Reus et al.[17] and Shimada[12].

In this study, the PI, MI, and NBI values of the treated cells were also determined to investigate the cytotoxic effect of levofloxacin. No important difference was observed in PI, MI, and NBI values between the treated cells and control group in all treatments with levofloxacin. On the contrary to the studies reported cytotoxic effect of levofloxacin on FLS[21] and rabbit ACL cells[22], in this study it was determined that levofloxacin did not show cytotoxic activity in human peripheral lymphocytes. Consequently, obtained data demonstrated that levofloxacin is not cytotoxic in human peripheral lymphocytes but could be genotoxic.

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