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Research Article

EFFECTS OF CIPROFLOXACIN ON GROWTH OF HUMAN RHABDOMYOSARCOMA (RD) AND RAT EMBRYO FIBROBLASTS (REF) CELL LINES: *IN VITRO* STUDY

Nada N. Al-Shawi*

Assistant Prof. Department of Pharmacology and Toxicology, College of Pharmacy, University of Baghdad, Baghdad- Iraq

Email: nadaalshawi@yahoo.com

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ABSTRACT

The possible anti-proliferative effects of ciprofloxacin utilizing cell lines obtained from different sources [human rhabdomyosarcoma (RD) and transitional rat embryo fibroblasts (REF) – (passage89)] were studied. The present study was carried out from January 2011 to May 2011.

Each of cell lines mentioned above was exposed to various concentrations of ciprofloxacin at concentrations (from 62.5 to 1000 mcg/ml) for 48hours in addition to control (zero) concentration. Four replicates were used for each data point and the results were expressed as mean ± SD. Ciprofloxacin caused significant growth inhibition on both cell lines only at 1000 mcg/ml concentration; but does not exert *in vitro* inhibitory effect on either human rhabdomyosarcoma (RD) or transformed rat embryo fibroblasts (REF) when assayed at concentrations of less than 1000 micrograms/ml.

Keywords: growth inhibition, ciprofloxacin, RD cell lines, REF cell lines, *in vitro* study

INTRODUCTION:

Ciprofloxacin, a fluoroquinolone antibiotic, is highly effective drug with a broad antibacterial spectrum. It is a bactericidal drug acts by inhibiting DNA gyrase and topoisomerase IV, which are essential enzymes in the reproduction of bacterial DNA [1]. However, it has been shown that this drug also inhibits mammalian topoisomerase II enzyme, a mitochondrial isoform which in turn leads to impairment of mitochondrial DNA (mit DNA) handling and subsequent gradual decrease in mit DNA contents in cells treated with the drug [2].

Rhabdomyosarcoma (RD), a cancer of connective tissues, in which the cancer cells are thought to arise from skeletal muscle progenitors. It can also be found attached to muscle tissue, wrapped around intestines, or in any anatomic location. Most occur in areas naturally lacking in skeletal muscle, such as the head, neck, and genitourinary tract [3].

Rat embryo fibroblast cell line (REF) was considered the most important source for the undifferentiated fibroblastic culture [4].

Conflicting data were obtained from studies performed by authors concerning the *in vitro* cytotoxic effects produced by different concentrations of ciprofloxacin on various cell lines, where number of investigators demonstrated the concentration-dependent anti-proliferative effects of the drug while others did not [5, 6].

Thus, the aim of the present study is to investigate the effects of different concentrations of ciprofloxacin on the growth of two cell lines obtained from either human or rat sources, [human rhabdomyosarcoma (RD) and transitional rat embryo fibroblasts (REF) – (passage 89)].

MATERIALS AND METHODS

Chemicals

Ciprofloxacin lactate injection (equivalent to 2000 mcg/ml ciprofloxacin base) used in the present study as stock solution, which was utilized to prepare different concentration (1000, 500, 250, 125 and 62.5 mcg/ml) of the drug. Other chemicals were of analytical grade.

Cell lines

Both human rhabdomyosarcoma and the transformed rat embyo fibroblast cell line were obtained from Iraqi Center for Cancer and Medical Genetic Researches (*ICCMGR*). They were maintained in RPMI-1640 medium supplemented with 10% heat-inactivated fetal calf serum, benzyl penicillin solution, streptomycin, nystatin and Sodium bicarbonate (4.4%) with a final pH of 6.8-7.2 at 37°C in a humidified incubator with 5% CO_2.

Cytotoxicity assay

RD and REF cells, cultured as described above, were seeded on micro-titration (96-well plates (at a concentration of $1X10^4$cells/well) in quadruple, and various concentrations of ciprofloxacin (from 62.5 to 1000mcg/ml) were added. The cells were incubated for 24h at 37°C in 5% CO_2 atmosphere. For the last 3 h of incubation, crystal violet stain 200µl in phosphate-buffered saline (PBS) was added to each well. The plates were incubated for 20 minutes at 37°C. After incubation, excess dye removed by washing the well three times with PBS.

The optical density of each well was read by using a micro-ELISA reader at a transmitting wavelength on 492nm with a spectrophotometer (ELIZA multi-well Reader, Organon-teknica, Austria) [7, 8].

The percentage of growth inhibition was calculated according to the following equation [9]:

$$IR\ \% = \frac{A - B}{A} \times 100$$

IR=inhibition rate, A= the optical density of control (zero) concentration, B= the optical density of each drug concentration.

Furthermore, digitalized camera was utilized to take pictures concerning the possible changes in growth of each cell line exposed to ciprofloxacin and compare that with cells not exposed to the drug.

STATISTICAL ANALYSIS

Data were analyzed by 2-way analysis of variance with ANOVA. Data are presented as means ± SD. The level of significance ($p<0.05$) was used for analysis of variance in all results presented in this study.

RESULTS

Table 1 demonstrated the results of various concentrations (from 62.5 to 1000) of ciprofloxacin each applied to RD cell

lines for 48hrs. There was no significant growth inhibition provoked by each ciprofloxacin concentrations (62.5, 125, 250 and 500 mcg/ml) ($p>0.05$); while, there was a significant growth inhibition observed at 1000mcg/ml drug concentration ($P<0.05$) on the RD cell line compared to control group (0 µg/ml). The percent of growth inhibition was 86.6%. Table 1.

Moreover, there was a manifestation of feature loss of RD cell line exposed to 1000mcg/ml ciprofloxacin for 48hrs with marked decrease in viability cell number of the intended cell line (figure 2) in comparison with confluent monolayer and cohesive cells concerning RD before exposure to 1000mcg/ml ciprofloxacin. Figure (1).

Table 1: The effects of different concentration of ciprofloxacin on growth of human rhabdomyosarcoma (RD) cell line.

Concentrations (mcg/ml)	Inhibition Rate (Mean ± SD)	% of growth Inhibition
Control (0)	0.362±0.047	-------
Ciprofloxacin 62.5	A, * 0.585±0.04	-61.6
Ciprofloxacin 125	A, * 0.605±0.0575	-67.1
Ciprofloxacin 250	A, * 0.453±0.0507	-25.1
Ciprofloxacin 500	A, * 0.558±0.04510	-54.1
Ciprofloxacin 1000	B, * 0.0485±0.002	86.6

-Non-identical capital letters (A, B) are considered significant ($P<0.05$) among RD cell lines exposed to different concentrations of ciprofloxacin.

* $P<0.05$: significant difference compared to un-treated cells (control).

Negative results of the % growth inhibition indicates proliferation.

Positive results of the % growth inhibition indicates anti-proliferation.

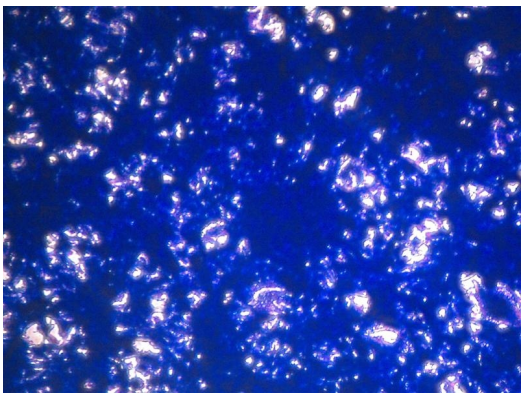

Figure 1 showing the cohesive cells of Rhabdomyosarcoma (RD) un-exposed to 1000mcg/ml ciprofloxacin. (Normal)

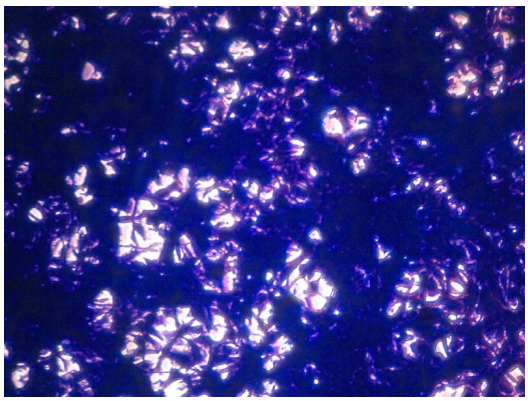

Figure 2 showing a manifestation of feature loss of RD cell line exposed to 1000mcg/ml ciprofloxacin for 48hrs with marked decrease in viability cell number

Similar to the above results, each concentration of ciprofloxacin utilized in this study (62.5, 125, 250 and 500mcg/ml) did not produce significant ($P>0.05$) growth inhibition on REF cell line exposed for 48hrs to drug; but there was a significant growth inhibition provoked by ciprofloxacin at 1000mcg/ml ($P<0.05$), where the % of growth inhibition was 81.72 % as shown in table 2.

Table 2: The effects of different concentration of ciprofloxacin on growth of rat embryo fibroblast (REF) cell line

Concentrations (mcg/ml)	Inhibition rate (Mean ± SD)	% of growth inhibition
Control (0)	0.279 ± 0.005	----------
Ciprofloxacin 62.5	A, * 0.334±0.0195	-19.7
Ciprofloxacin 125	A, * 0.338±0.0194	- 21.15
Ciprofloxacin 250	A, * 0.395±0.0215	- 41.57
Ciprofloxacin 500	A, * 0.555±0.0925	- 98.92
Ciprofloxacin 1000	B, * 0.051±0.0434	81.72

Non-identical capital letters (A, B) are considered significant ($P<0.05$) among REF cell lines exposed to different concentrations of ciprofloxacin.

* $P<0.05$: significant difference compared to untreated cells (control).

Negative results of the % growth inhibition indicates proliferation

Positive results of the % growth inhibition indicates anti-proliferation

Additionally, a manifestation of feature loss of the REF cell line exposed to 1000mcg/ml ciprofloxacin for 48hrs with marked decrease in viability cell number of the intended cell line were observed in figure 4 in comparison with confluent monolayer and cohesive cells concerning REF before exposure to 1000mcg/ml ciprofloxacin. Figure (3).

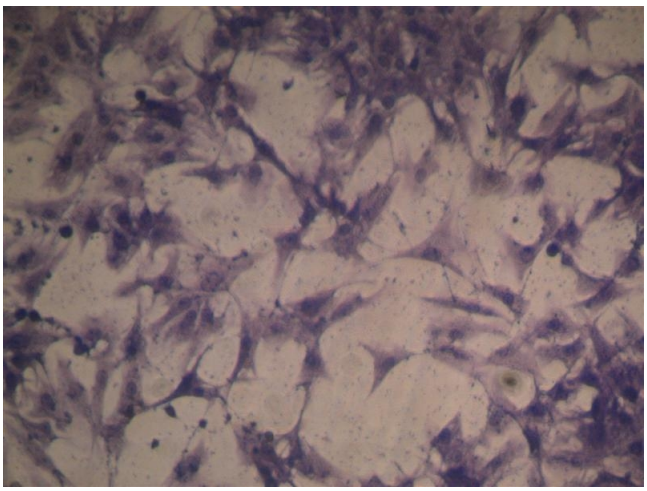

Figure 3 showing the cohesive cells of rat embryo fibroblast (REF) before application of 1000mcg/ml ciprofloxacin (Normal)

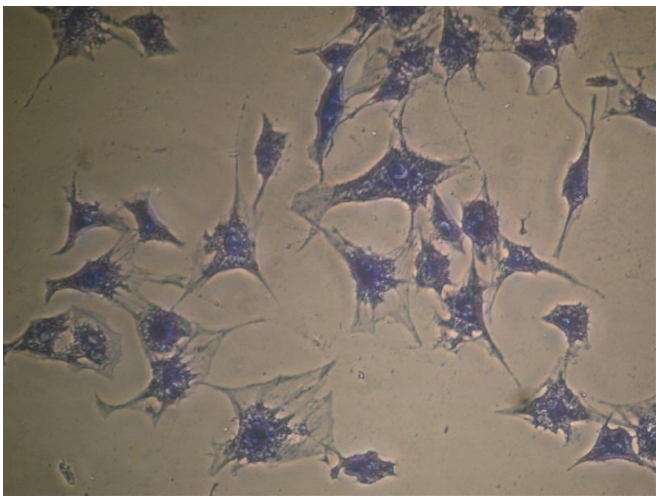

Figure 4 showing a manifestation of feature loss of RD cell line exposed to 1000mcg/ml ciprofloxacin for 48hrs with marked decrease in viability cell number

DISCUSSION

There were limited *in vitro* studies concerning the anti-proliferative effect of various concentrations of fluoroquinolones antibiotics especially, ciprofloxacin where a significant growth inhibition has been observed in a variety of human tumor cells, such as human leukemic cells, osteoblast-like MG-63 human osteosarcoma cells, and transitional cell carcinoma of the bladder[10 ,11 ,12]. In addition, it was revealed that ciprofloxacin had growth-inhibitory effects against human transitional cell carcinoma of the bladder cell lines, TCCSUP, T24 and J82 *in vitro* [13]. In the previous reports, the concentration-dependent anti-proliferative effect of fluoroquinolones on various cell lines was demonstrated. The present study revealed that human rhabdomyosarcoma (RD) or rat embryo fibroblasts (REF) cell lines was exposed to ciprofloxacin for 48hrs produced growth inhibition but the anti-proliferative effect of the drug was only observed at 1000 mcg/ml concentration, (not less than this concentration), where the growth inhibition of the former cell lines was 86.6% and 81.1% for the later cells; thus, the results obtained from this study was inconsistent with the previous reports. This probably may be due to the different property of each cell lines used in this study compared to those in the previous studies. Moreover, it was demonstrated that the anti-proliferative potential of ciprofloxacin against other type of cell lines (human bladder cells) varies according to drug concentration and time of incubation [14].

This inhibitory action of (1000mcg/ml) ciprofloxacin can be attributed either to the sensitivity of both cell lines to the cytoxic activity of the drug or may be due to the modulation properties exerted by the drug, where, many authors demonstrated that fluoroquinolones antibiotics provoke down regulation of the synthesis of pro-inflammatory cytokines TNF-α, IFN-γ and IL-8[15, 16]. The morphological features observed in this study revealed a feature loss with marked decrease in viability cell number when the intended cell lines were exposed to 1000 mcg/ml ciprofloxacin for 48hrs in comparison with confluent monolayer and cohesive cell of control cell, further suggesting that these cell lines were sensitive to the action of the higher concentration of ciprofloxacin used in this study.

The results of this study provide an evidence, for the first time, to our knowledge on how various concentrations of ciprofloxacin have different actions on cellular growth, where 1000mcg/ml concentration of the intended drug induced growth inhibition when applied to RD and REF cell lines, while less than that concentration (62.5-500 mcg/ml) each was failed to induce such an effect; accordingly, higher concentration of ciprofloxacin may prove useful as a potential preventive and/or therapeutic agent as an anticancer agent.

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