Research Article

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IN VITRO EFFECTS OF CEFTRIAXONE ON GROWTH OF HUMAN RHABDOMYOSARCOMA (RD) AND ON RAT EMBRYO FIBROBLASTS (REF) CELL LINES

Nada N. Al-Shawi*

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

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*Corresponding author

E-mail: nadaalshawi@yahoo.com

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ABSTRACT

Many reports demonstrated that antibiotics like cefazoline, ciprofloxacin, trimethoprim-sulfamethoxazole and others have the ability to inhibit growth of various cell lines. Thus, this study was designed to investigate whether or not ceftriaxone may possess cell growth inhibition using *In vitro* study and utilizing two cell lines (human rhabdomyosarcoma (RD) and rat embryo fibroblasts (REF)). Various concentrations of Ceftriaxone (62.5, 125, 250, 500 and 1000 mcg/ml) were utilized in this study. The drug relatively caused concentration-dependent inhibition on growth of the intended cell lines used in this study, where, the growth inhibition induced by the drug was statistically significant at 125mcg/ml and above. The results obtained from this work encourage further study of the possibility of clinical application of ceftriaxone to prevent the occurrence of different tumors.

Keywords: *In vitro* study, ceftriaxone, RD cell lines, REF cell lines, Growth inhibition

INTRODUCTION

Ceftriaxone, a bactericidal 3rd generation cephalosporin, belongs to β -lactam group of antibiotics. It has broad spectrum activity where it acts by inhibiting bacterial cell wall of many Gram-positive and Gram negative bacteria¹. Rhabdomyosarcoma (RD), a cancer of connective tissues, in which the cancer cells are thought to arise from skeletal muscle progenitors. Mostly occur in areas naturally lacking in skeletal muscle, such as the head, neck, and genitourinary tract². Rat embryo fibroblast cell line (REF) was considered the most important source for the undifferentiated fibroblastic culture³. Many reports demonstrated that antibiotics like cefazoline, ciprofloxacin, trimethoprim-sulfamethoxazole and others have the ability to inhibit growth of various cell lines^{4,5}. Thus, this study was designed to investigate whether or not ceftriaxone may possess cell growth inhibition using *in vitro* study and utilizing two cell lines, human rhabdomyosarcoma (RD) and transitional rat embryo fibroblasts (REF).

MATERIALS AND METHODS

Chemicals

Ceftriaxone vial 1193 mg ceftriaxone disodium salt (equivalent to 1000 mg ceftriaxone base as dry powder for injection, was freshly reconstituted in 10 ml distilled water and used in the present study as stock solution), and it 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 embryo 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% CO₂.

Cytotoxicity assay

RD and REF cells, cultured as described above, were seeded on micro-titration (96-well plates at a concentration of 1X10⁴cells/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% CO₂ atmosphere. For the last 3 h of incubation, crystal violet stain 200 μ l 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 (ELISA multi-well Reader, Organon-tekhnica, Austria)^{6,7}.

The percentage of growth inhibition was calculated according to the following equation⁸.

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

IR=inhibition rate,

A=optical density of control (zero) concentration, B=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 ceftriaxone and compared 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 $\pm$ SD. The level of significance (p<0.05) was used for analysis of variance in all results presented in this study.

Table 1: The effects of different concentration of ceftriaxone on growth of human Rhabdomyosarcoma (RD) cell line

Concentration mcg/ml	Inhibition rate (mean±SD)	% of Growth Inhibition
Control (0)	0.362±0.047	-----
Ceftriaxone 62.5	A : 0.386±0.0174	-6.629
Ceftriaxone 125	B, * : 0.236±0.0453	34.806
Ceftriaxone 250	C, * : 0.05±0.0051	86.187
Ceftriaxone 500	B, * : 0.1133±0.0393	68.701
Ceftriaxone 1000	B, * : 0.0475±0.002	86.87

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

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

- Negative results of the % growth inhibition indicate proliferation.

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

Table 2: The effects of different concentration of ceftriaxone 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	-----
Ceftriaxone 62.5	A, * : 0.361±0.0195	-29.391
Ceftriaxone 125	B, * : 0.226±0.0194	18.996
Ceftriaxone 250	B, * : 0.106±0.0215	62.007
Ceftriaxone 500	B, * : 0.113±0.0925	59.498
Ceftriaxone 1000	C, * : 0.054±0.0043	80.645

- Non-identical capital letters (A, B and C) are considered significant ($P < 0.05$) among REF 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: Cohesive cells of Rhabdomyosarcoma (RD) un-exposed to 1000 mcg/ml ceftriaxone

Figure 2: Manifestation of feature loss of RD cell line exposed to 1000mcg/ml ceftriaxone for 48 h with marked decrease in viability cell number

Figure 3: Cohesiveness of Rhabdomyosarcoma (RD) cells un-exposed to 1000mcg/ml ceftriaxone

Figure 4: Manifestation of feature loss of REF cell line exposed to 1000mcg/ml ceftriaxone for 48hrs with marked decrease in viability cell number

RESULTS

Table 1 demonstrated the results of various concentrations (from 62.5 to 1000) of ceftriaxone each applied to RD cell lines for 48hrs. There was no significant growth inhibition provoked by 62.5mcg/ml ceftriaxone concentration compared to cells not exposed to the drug ($P>0.05$); while, there were significant growth inhibition observed at 125, 250, 500 and 1000mcg/ml drug concentration ($P<0.05$) on the RD cell line compared to cells not treated with the drug (0 $\mu\text{g/ml}$). The percent of growth inhibition were 34.806, 86.187, 68.701 and 86.87%, respectively. (Table 1)

Concerning morphological features of RD cell lines, figure 2 demonstrated that maximum concentration of ceftriaxone (1000mcg/ml) used in this study caused feature loss of RD cell line exposed to the drug for 48hrs with marked decrease in viability cell number of the intended cell line in comparison with confluent monolayer and cohesiveness of RD before exposure to such concentration of the drug. (Figure 1)

The results of this study demonstrated that the intended concentrations of ceftriaxone caused similar effects which were observed concerning REF cell lines; where, there was no significant growth inhibition provoked by 62.5 mcg/ml ceftriaxone concentration compared to cells not exposed to the drug ($p>0.05$); while, there were significant growth inhibition observed at 125, 250, 500 and 1000mcg/ml drug concentration ($p<0.05$) on the REF cell line compared to cells not treated with the drug (0 $\mu\text{g/ml}$). The percent of growth inhibition were 18.996, 62.007, 59.498 and 80.645%, respectively. (Table 2)

Concerning morphological features of REF cell lines, figure 4 demonstrated that maximum concentration of ceftriaxone (1000 mcg/ml) used in this study caused feature loss of REF cell line exposed to the drug for 48 h with marked decrease in viability cell number of the intended cell line in comparison with confluent monolayer and cohesiveness of RD before exposure to such concentration of the drug. (Figure 3)

DISCUSSION

Many authors have been reported that antibiotics like cefazoline, ciprofloxacin, co-trimoxazole and moxifloxacin have the ability to inhibit growth utilizing various cell lines^{4, 5}. But, there was no *In vitro* study demonstrating the effect of different concentrations of ceftriaxone on growth of cell lines.

The present study revealed that when human rhabdomyosarcoma (RD) or rat embryo fibroblasts (REF) cell lines was exposed to ceftriaxone, a beta lactam antibiotic for 48 hrs produced growth inhibition relatively in concentration-dependent manner (from 125mcg to 1000mcg/ml). By comparing the result of this study with the other one which was performed on other drug, the growth inhibitory action of various concentrations of ceftriaxone on the previously-mentioned cell lines can be attributed to the sensitivity of both cell lines to the cytotoxic activity of the drug⁹.

Cephalosporins are beta lactam-containing compounds, used for treatment of bacterial infection¹⁰. They have also been used in selective targeting of anticancer compounds

to tumor cells utilizing antibody-directed enzyme prodrug therapy (ADEPT)¹¹⁻¹⁵.

In addition, there are many types of antibiotics (e.g. anthracyclines, bleomycin) that have been used to treat cancer. However, research into the possibility of utilizing beta-lactam antibiotics as potential anticancer medications has been relatively non-existent. It has been demonstrated that beta-lactams could play a role as anticancer drugs^{16, 17}.

The results of this *In vitro* study may provide an additional evidence for the effect of various concentrations of ceftriaxone on growth of two cell lines, (RD and REF) used in this study, where a concentration-dependent anti-proliferative effects of ceftriaxone were observed; thus, ceftriaxone may prove useful as a potential preventive and/or therapeutic agent as an anticancer agent. Further studies are needed to completely assess the antiproliferative effect of the drug utilizing *In vivo* model.

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