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## DRUG BIOCHEMISTRY

# THE INFLUENCE OF CIPROFLOXACIN ON HAMSTER OVARIAN CANCER CELL LINE CHO AA8

TOMASZ KLOSKOWSKI[1], JOANNA OLKOWSKA[1], AYBARS NAZLICA[1] and TOMASZ DREWA[1,2]

[1] Department of Tissue Engineering, Chair of Mmedical Biology, Nicolaus Copermicus University, Karłowicza 24, 85-092 Bydgoszcz, Poland
[2] Department of Urology, Nicolaus Copermicus University, M. Curie Skłodowskiej 9, 85-094 Bydgoszcz, Poland

**Abstract:** Ciprofloxacin is a chinolone antibiotic, which is used mainly in the treatment of urinary tract infections but also in pulmonary tract, prostate gland, bone and bone marrow infection. Ciprofloxacin is also known for its anticancer *in vitro* properties. In this study hamster ovarian cancer line CHO AA8 was used for evaluation of cytotoxic properties of ciprofloxacin against neoplastic cells. For this purpose we used different concentrations of ciprofloxacin range from 10 to 1000 µg/mL. Cell viability was counted using trypan blue assay. Ciprofloxacin induced morphological changes and decreased viability in a concentration and time dependent manner within CHO AA8 cells. In low concentrations cytotoxic effect of ciprofloxacin is weak only after 24 h incubation. In the highest concentration of ciprofloxacin, after 24, 48 and 72 h incubation only a very small number of living cells (not exceeding 1%) was observed. No living cells were observed after 96 h of incubation times and ciprofloxacin concentrations of 800 and 1000 µg/mL. These promising results deserved future studies on chinolones and ovarian cancer.

**Keywords:** ciprofloxacin; ovarian cancer, *in vitro*; apoptosis

Ovarian cancer (OCA) is the most lethal cancer of reproductive tract in female. According to the National Cancer Institute, OCA is in the USA the eighth most common and the fifth cause of cancer death in women (globally the seventh) and about 204.000 women are afflicted with OCA worldwide each year (1, 2). Around 14.000 women develop breast or ovarian cancer in Poland every year (3). Ovarian cancer is usually treated with platinum-based chemotherapy that includes a platinum-based drug (carboplatin or cisplatin) and a taxane (paclitaxel or docetaxel) (4–6).

Systemic therapy used in the management of ovarian cancer lead to disease progression very often, that is why it is necessary to search for new drugs with better therapeutic index for neoadjuvant and adjuvant treatments.

Ciprofloxacin is a chemotherapeutic agent that belongs to fluoroquinolones (7). This group of antibiotics has a broad spectrum of action against pathogens, especially Gram(-) aerobic bacilli, thanks to inhibition of bacterial gyrase (8). Ciprofloxacin also inhibits topoisomerase II in eukaryotic, including mammalian cells (9, 10). Ciprofloxacin is also known for its anticancer properties enabling cell cycle arrest and creating double-strand breaks in nucleic acid, which triggers apoptosis of cancer cells *in vitro* (11, 12). Ciprofloxacin inhibits activity of the topoisomerase II in high concentrations, low concentrations not always lead to the enzyme inhibition but cells proliferation is stopped. Therefore it must be additional mechanisms responsible for ciprofloxacin cytotoxicity which can be connected with its action at the cell membrane and mitochondria levels (13). Ciprofloxacin has the ability to inhibit cells proliferation thanks to mitochondrial DNA damage (14). Ciprofloxacin reacts with the mitochondrial topoiso-

* Corresponding author: phone: +48792791314; fax: +48525853742, e-mail: tomaszkloskowski@op.pl

merase II isoform. Thanks to that, ciprofloxacin is able to inhibit mtDNA synthesis with subsequent mitochondria damage, which leads up to the reduction of the intracellular ATP amount with subsequent apoptosis (14, 15). Ciprofloxacin-induced cytotoxicity in cells may be a result of free radicals generation. According to the *in vitro* and *in vivo* studies, ciprofloxacin phototoxicity during UVA irradiation was caused by arising reactive oxygen species such as hydrogen peroxide and hydroxylic radical (16–18). Ciprofloxacin induced oxidative stress in human fibroblasts and in cerebral and hepatic tissues of rats *in vivo* (19, 20).

## EXPERIMENTAL

### Cell line

CHO AA8 line is an hamster ovarian cancer cell line. These are epithelial-like cells and they grown in monolayer culture. Growth of CHO AA8 cell line is dependent on the attachment to the culture bottle.

CHO AA8 cell line was obtained from Department of Histology and Embryology from Collegium Medicum in Bydgoszcz thanks to courtesy of prof. Alina Grzanka. CHO AA8 line was cultured in DMEM/HAM'S F-12 medium containing 10% fetal bovine serum (FBS), supplemented with 5 µg/mL amphotericin B, 100 µg/mL streptomycin and 100 U/mL penicillin. This cell line were grown in plastic tissue culture T-flasks 25 $cm^2$ (Nunc) at 36$^o$C and 5% $CO_2$.

### Drug

Stock solution of ciprofloxacin (Polfa Warszawa, Poland) was 10 mg/mL. Final concentrations of 25 – 1000 µg/mL were produced by diluting initial concentration in complete growth medium prepared for CHO AA8 cells.

### Cytotoxic influence of ciprofloxacin on CHO AA8 cell line

Cells were seeded on 24-well plates at a density of 5 x $10^4$ cells on well. Cells were allowed to adhere for 24 hours and were then exposed on ciprofloxacin. CHO AA8 cells were exposed on various concentrations of ciprofloxacin (10, 25, 50, 100, 200, 500, 800, and 1000 µg/mL). Cells were exposed on ciprofloxacin through 24, 48, 72, and 96 hours. After each time of incubation, medium with antibiotic was removed from wells, each well was flushed with 0.5 ml PBS and then cells were detached from wells using 0.5 mL 0.05% trypsin. After centrifugation at 300 × g for 5 min. cells were suspended in 1 mL of medium.

Viability was based on the presence of living cells. After trypsinization and centrifugation of cells to 50 µL of the cells solution was taken and mixed-up with the same volume of trypan blue. Trypan blue dye is commonly used to estimate cell viability. Cell counting after fixation with alcohol without any stain can be even used in assessing cell viability. Using trypan blue assay we were able to counted all the cells from the sample, i.e. cells growing in monolayer and detached cells as well. We don't used MTT assay, which is a method to examine cell proliferation within monolayers. MTT method is not appropriate, when detached cells have to be taken into account. The medium with detached cells over monolayer is routinely removed before MTT will be added. The percentage of alive cells within the population of detached cells is presumed to be as high as 30% (21–23). There is a probability that cancer stem cells can be found within the population of detached cells (24). And finally, we used trypan blue assay, because we focused more on the examination of cell viability than cell proliferation. Cells were counted in Neubauer chamber under the inverted microscope at 100x magnification. Application of the dye has enabled distinguishes living cells from the dead cells. The number of living cells was calculated using the formula:

$$\frac{A}{4} \times 2 \times 10^4 \times B$$

A – number of cells counted in Neubauer chamber at 100× magnification

B – mL of volume in which cells were suspended

Each concentration, together with control, was counted five times. From obtained results, for each concentration average value was calculated. The cytotoxicity of ciprofloxacin against ovarian cancer cell line was established on the basis of a comparison of viability of the cells in the various concentrations of ciprofloxacin to lifetime of cells derived from control and calculated from the formula:

$$X = \frac{L_c}{L_k} \times 100\%$$

where X = cytotoxicity of ciprofloxacin, $L_c$ = the average number of cells in the test sample,

$L_k$ = the average number of cells in control

Each result was calculated from three independent measurements. Results were presented as the means ± standard deviations. The means were compared using *t*-Studnent test. Values of $p < 0.05$ were considered significant.

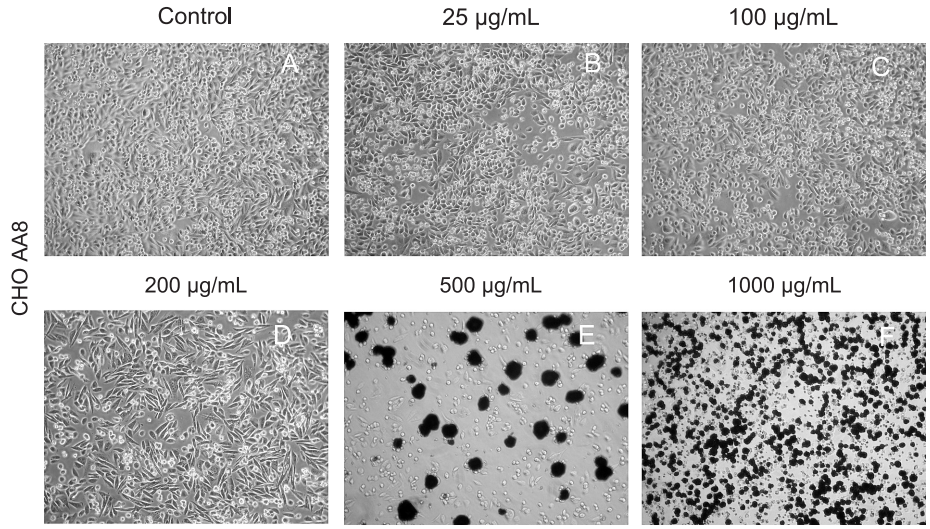


Figure 1. Morphology of CHO AA8 cell line after 24 h incubation with ciprofloxacin

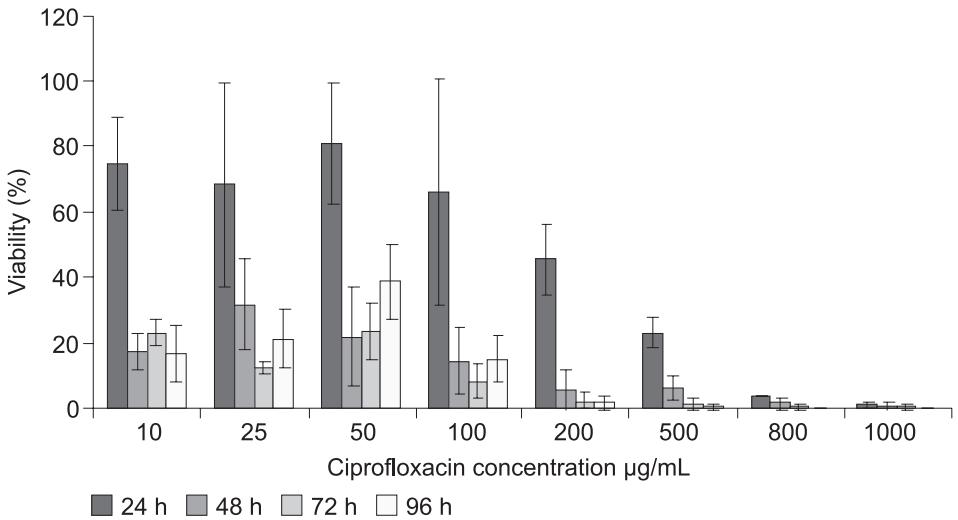


Figure 2. Viability of CHO AA8 cells after treatment with ciprofloxacin

## RESULTS

### Morphological changes after incubation with ciprofloxacin

Ovarian cancer cell line in the control had a regular shape and size (Fig 1A). Line CHO AA8 was elongated and spindle-shaped, cells tightly covered T-flask area with numerous cytoplasmic lamellipodia (Fig. 1A, B, C). After incubation with ciprofloxacin CHO AA8 cells lost their regular shape and size, they lost their cell-cell contact (Fig 1E, F). Many cells lost their attachment to surface of culture flask, a majority of cells were rounded in appearance what suggested that the cell death process was going on (Fig. 1E, F).

### Cytotoxic influence of ciprofloxacin on viability of CHO AA8 cell line

Ciprofloxacin cytotoxicity on CHO AA8 cell line after each incubation times is presented in Fig. 2 (the means ± SD). CHO AA8 cell line viability decreases with increasing concentration of

chemotherapeutic agent. In low concentrations (10 – 100 µg/mL) cytotoxic effect of ciprofloxacin is weak only after 24 h incubation. After each incubation times, in low concentrations, cells may even surpass the number of cells from lower concentrations. In concentration of 200 µg/mL viability of cells decreased under 10% after 48, 72, and 96 h incubation time. In the highest concentration of ciprofloxacin, after 24, 48 and 72 h incubation only a very small number of living cells (not exceeding 1%) was observed. No living cells were observed after 96 h of incubation times and ciprofloxacin concentrations of 800 and 1000 µg/mL ( Fig. 2).

**Discussion**

Our results showed that ciprofloxacin is cytotoxic against CHO AA8 cells. Ciprofloxacin induced morphological changes in CHO AA8 cells and decreased their viability in *in vitro* conditions.

There are no similar publications about effect of ciprofloxacin on survivability of the ovarian cancer cells. However, the results of this study are similar to those receied by Mondal et al. on the NCI-H460 lung cancer line (25). In this study ciprofloxacin was used in concentration of 40 µg/mL through 24, 48, 72 and 96 h incubation times. It was observed a decrease in viability related with dose of ciprofloxacin and incubation time. Additionally, we observed similar to mentioned above morphological changes which occurred after ciprofloxacin exposition. Percent of apoptotic cells after ciprofloxacin treatment increased in time dependent manner. In the study of Mondal et al. ciprofloxacin induced apoptotic cell death, inhibited proliferation of cells, increased population doubling time and reduced saturation density. Described alterations in cells, which results from the action of ciprofloxacin, are probably a consequence of topoisomerase II inhibition by this antibiotic.

Ciprofloxacin also shows cytotoxic properties on other cancer cell lines *in vitro*.

After oral administration of ciprofloxacin, there is higher concentrations of drug in urine than in serum, that's why most research are conducted into the use of potential anticancer activities of ciprofloxacin against bladder cancer cells. There was examined influence of ciprofloxacin on different human bladder cancer cell lines: TCCSUP, T24, J82, HTB9, MBT-2 and human urothelial cancer cell lines HT 1197 and HT 1376 (26–30). Ciprofloxacin causes viability decrease in dose- and time-dependent manner in these cell lines, inhibition of proliferation, cell cycle arrest and induction of apoptosis. Similar results were observed in study on three colorectal cancer cell lines (CC-531, SW-403, HT-29) and on androgen independent prostate carcinoma PC3 cells. It has to be emphasized that ciprofloxacin has not cytotoxic effect on normal prostate epithelial cells (MLC8891 cell line) (31, 32).

Influence of ciprofloxacin on acute T-cell leukemia cell line (Jurkat) resulted in inhibition of their proliferation, reduction of the mitochondrial membrane potential and decrease in the activity of the respiratory chain. There were also observed reduction in content of the mtDNA and decreased influx of $Ca^{2+}$ into Jurkat cells (33).

Segev et al. were studied administration of ciprofloxacin to female pelvic tissue after oral administration. In this work 25 women received one dose of 500 mg ciprofloxacin before surgery. This study showed that 9 h after administration of drug, concentration of ciprofloxacin in serum reached value above 0,5 µg/mL. In case of ovarian tissue 6-9 h after administration ciprofloxacin concentration exceeded 0,5 µg/mL and after 11–12 h this concentration levels rose. In case of serum and female pelvis tissue at 11–12 h levels were of the same magnitude At 6–9 h, when tissue levels were the highest, the ratio between ovarian tissue and serum was 1.08 (34). This work indicate, that ciprofloxacin reach higher concentration in ovarian tissue than in the serum after oral administration. Our results show that dose of 10 µg/mL after 48 h incubation with ciprofloxacin can decrease viability of ovarian cell line to 17,5% compared to control. Together these results indicate, that ciprofloxacin after prolonged use may be effective in anticancer therapy against ovarian cancer.

Our results support the suspicion that ciprofloxacin could be tested as possible neoadjuvant or adjuvant therapy for ovarian cancer. Three properties of ciprofloxacin give confirmation of this thesis. Ciprofloxacin is toxic against ovarian cancer cell lines in a concentration and time dependent manner. Ciprofloxacin inhibits topoisomerase II, which leads to cell death by apoptosis in malignant but not in normal cells. Ciprofloxacin accumulates in ovarian tissue after oral administration. Future experiments are needed to investigate ciprofloxacin activity against ovarian cancer *in vivo*.

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