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R. KOZIEL[1], J. SZCZEPANOWSKA[1], A. MAGALSKA[2], K. PIWOCKA[2], J. DUSZYNSKI[1], K. ZABLOCKI[3]

CIPROFLOXACIN INHIBITS PROLIFERATION AND PROMOTES GENERATION OF ANEUPLOIDY IN JURKAT CELLS

[1]Laboratory of Bioenergetics and Biomembranes, Nencki Institute of Experimental Biology, Warsaw, Poland;
[2]Laboratory of Molecular Bases of Aging, Nencki Institute of Experimental Biology, Warsaw, Poland;
[3]Laboratory of Cellular Metabolism, Nencki Institute of Experimental Biology, Warsaw, Poland

Ciprofloxacin is widely used in antimicrobial therapy. However it also inhibits mitochondrial topoisomerase II and therefore affects cellular energy metabolism. At a concentration exceeding 80 μg/ml ciprofloxacin induces apoptosis, while at 25 μg/ml it inhibits proliferation of Jurkat cells without any symptoms of cell death. The aim of this study was to explain the mechanisms of ciprofloxacin-evoked perturbations of the cell cycle. Human lymphoidal cells (Jurkat) were exposed to ciprofloxacin (25 μg/ml) for 4-11 days and effects of the drug on cell proliferation (light microscopy), cell cycle (flow cytometry), cell size and morphology (confocal microscopy) as well as number of chromosomes (chromosomal spread analysis) were investigated. Exposition of Jurkat cells to ciprofloxacin inhibited cell proliferation, increased proportion of cells in the G2/M-phase of the cell cycle, compromised formation of the mitotic spindle and induced aneuploidy. These observations indicate that ciprofloxacin applied at concentrations insufficient for induction of apoptosis may stop cell proliferation by inhibition of mitosis. Chromosomal instability of such cells may, at least potentially, increase a risk of cancer development.

Key words: *aneuploidy, cell cycle, cell death, cell proliferation, ciprofloxacin, Jurkat cells, karyotype, chromosomal instability*

INTRODUCTION

Ciprofloxacin belongs to the class of 4-fluoroquinolone antibiotics which are commonly used in therapy of many bacterial infections. Its antimicrobial activity is based on the inhibition of bacterial DNA gyrase. However, this drug has also been found to affect mammalian topoisomerase II, especially its mitochondrial isoform. This impairs mitochondrial DNA (mtDNA) handling and eventually results in a gradual decrease in mtDNA content in ciprofloxacin-treated cells (1, 2). As mtDNA encodes 13 proteins, all engaged in oxidative phosphorylation, it is obvious that aberrant expression of mitochondrial genes and/or partial depletion of mitochondria of mtDNA may affect mitochondrial energy metabolism. In fact, disturbance of mitochondrial respiration and ATP synthesis has been postulated to explain the cytotoxic effect of ciprofloxacin (2, 3). The gradual elimination of mtDNA content in ciprofloxacin-treated cells occurs in a concentration-dependent manner and begins when the concentration of the drug reaches ca. of 20 μg/ml. This effect needs a few days of culture to develop (2, 4). It is also noteworthy that ciprofloxacin applied at a concentration as high as 100 μg/ml does not affect nuclear topoisomerase II. Moreover, inhibition of the nuclear enzyme by much higher concentrations of the drug usually needs only a few hours to cause cell death (3). Thus, it may be assumed that in cells exposed to ciprofloxacin at 25 μg/ml (as applied in this paper) the effects observed are not related to an impairment of nuclear DNA handling. In the clinical use of ciprofloxacin as an antimicrobial agent its serum concentration never exceeds 7 μg/ml (5).

Interestingly, there are also well documented observations indicating that ciprofloxacin shows a substantial antiproliferative activity against various cancer cells. However, to achieve this an additional effect ciprofloxacin has to be used at a much higher concentration than that for the treatment of infectious diseases (6). Therefore, if used as an anticancer agent, ciprofloxacin would reach blood concentration higher than those during its antimicrobial administration. The pro-apoptotic and therefore potentially anticancer action of ciprofloxacin was found to be particularly efficient against bladder, colorectal and human prostate cancer cells (7-9). Ciprofloxacin has also been shown to affect proliferation of human osteosarcoma cells and leukemic cell lines *in vitro* (10, 11). Ciprofloxacin applied at high concentrations (200-300 μg/ml) effectively induced apoptosis of bladder carcinoma cells and led to cell cycle arrest at the S/G2 stage (8, 12). Taken together, these observations clearly indicate feasibility of trying ciprofloxacin in anticancer therapy.

The major aim of our previous studies was to characterize mtDNA-dependent effects of ciprofloxacin in Jurkat cells (4). As the effective degradation of mtDNA due to inhibition of mitochondrial topoisomerase II needs a few cell cycles to occur, it was crucial to use this drug at a concentration that was efficient for mtDNA degradation but not for induction of cell death. We found that an optimal drug concentration fulfilling these criteria was 25 μg/ml (4) and Jurkat cell grown in the presence of ciprofloxacin at this concentration for 11 days did not exert any symptoms of apoptotic cell death. It was evaluated

using annexin V/7-AAD test for visualization of phosphatidylserine on the surface of living cell (hallmark of apoptosis) and nuclei (test for necrosis) and measurement of caspase 3 activity. None of them indicated accelerated cell death. We also showed that ciprofloxacin applied at this concentration in the growth medium continuously for 4, 7 or 11 days caused a significant reduction in the mtDNA content, a decrease of the mitochondrial respiration rate and the mitochondrial inner membrane potential, a transient decrease in the cellular capability of ATP synthesis and inhibition of store-operated calcium entry (13). Thus, it was suggested that prolonged exposition of cells to ciprofloxacin may result in aberrant intracellular calcium signalling. Moreover, it was observed that the ciprofloxacin-evoked changes in cellular energy metabolism and calcium influx coincided with a decreased rate of cell proliferation. However, the potential link between those effects has remained unclear (4).

Therefore, the aim of the present study was to explain the effect of ciprofloxacin on Jurkat cells proliferation. As the final concentration of ciprofloxacin was only 25 μg/ml, it allowed maintaining the cells in the presence of the drug for a few days and avoiding the fast effects based on induction of cell death. We found that exposition of Jurkat cells to ciprofloxacin under such conditions results in aberrant formation of the mitotic spindle and promotes generation of aneuploidy. These results indicate, that although ciprofloxacin does not exert any significant side effects on cellular energy metabolism and cell proliferation if applied at the concentration corresponding to that for the antimicrobial treatment (Koziel, unpublished observation) and kills cells if used at a much higher concentration (above 80 μg/ml) (7, 9) its action at a concentration 25 μg/ml leads to chromosomal instability. It may, at least hypothetically, increase of a risk of cancer development.

MATERIAL AND METHODS

Chemicals

Ciprofloxacin was from ICN Biomedicals, Inc. (Aurora, OH, USA). Monoclonal anti-α tubulin antibody were from Sigma Chemicals (St. Louis, MO, USA). Other chemicals were of analytical grade.

Cell culture

Human lymphoblastoid T cells (Jurkat) were grown in RPMI-1640 medium supplemented with 2 mM glutamine (Gibco Invitrogen), 50 μM uridine, 10% fetal bovine serum (Gibco Invitrogen), penicillin (100 units/ml) and streptomycin (50 μg/ml) in a humidified atmosphere of 5% CO_2/95% air at 37°C. Where indicated, ciprofloxacin (25 μg/ml) was added. Cells were seeded every two days (to a density of $0.5x10^6$ cells per ml) and all experiments were conducted one day after seeding. Cell culture densities were evaluated after trypane-blue staining of cells using a Neubauer camera.

Cell cycle

Cell cycle was analyzed in fixed cells stained with propidium iodide. Two-milliliter samples of culture suspension containing $1x10^6$ cells were centrifuged at 200x g for 3 min and rinsed with 3 ml of PBS. After centrifugation, the cell pellet was suspended in 100 μl of PBS and transferred into tubes containing 3 ml of ice-cold 70% ethanol. Fixed cells were stored at -20°C until further analysis. Before measurement, the fixed cells were centrifuged (3 min at 240x g), rinsed with ice-cold PBS and centrifuged again. The resulting pellets were suspended in 0.5 ml PBS and then 0.5 ml of a mixture of 0,2 M phosphate buffer pH 7.4 and 0.05 M citrate buffer pH 7.4, 96: 4 was added. After 2 min of incubation in darkness, the cells were centrifuged for 4 min at 240x g and resuspended in 0.5 ml PBS containing propidium iodide (5 μg/ml) and RNAse A (100 μg/ml). After 30 min of incubation in darkness, the cell cycle profile was estimated using flow cytometry (Becton Dickinson). The proportion of cells at different phases of the cell cycle was calculated using ModFit software.

Visualization of nuclei, mitotic spindles and cell morphology

The cells were fixed in paraformaldehyde (4% solution in PBS, pH 7.4) for 20 min at room temperature. After triple rinsing with PBS supplemented with 10% FBS, cell suspension was centrifuged (3 min at 200x g) and incubated for 1 h at room temperature with anti-α tubulin primary monoclonal antibody suspended in PBS containing 10% FBS and 0.2% saponin. After incubation the cells were rinsed three times with PBS containing 10% FBS and incubated for 1 h in the same medium supplemented with 0.2% saponin and TRITC-coupled secondary antibody. Nuclei were labelled with DAPI (1 μM) and actin filaments with falloidine coupled with FITC. After labelling the cells were rinsed three times with PBS containing 10% FBS and once with PBS, spread on microscopic slides in Mounting Medium (DacoCytomation) and analyzed using confocal microscopy (Leica TCS SP2 Spectral Confocal and Multiphoton Microscope) with an oil immersion objective x 63.

Chromosome counting

Small sample of cell suspension was incubated for 4 h at 37°C in KaryoMax® Colcemid (Invitrogen) solution. Then, the cells were centrifuged for 5 min at 200x g. The cellular pellet was gently suspended in 1 ml of PBS and centrifuged again for 2 min at 3000x g. The pellet was suspended in a small amount (ca. 100 μl) of PBS, 0.9 ml of 75 mM KCl was added and the mixture was incubated for 17 min at 37°C. Next, a freshly

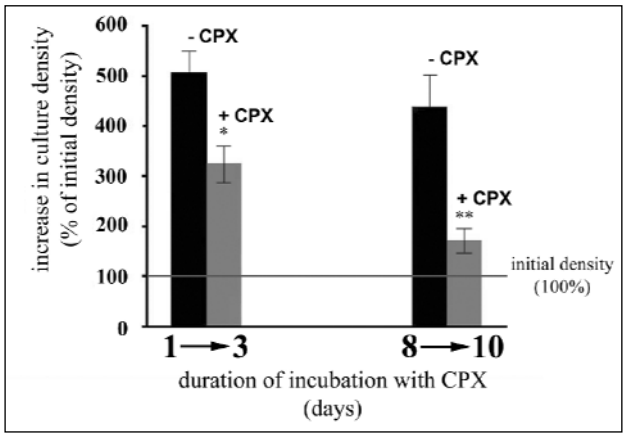

Fig.1. Effect of ciprofloxacin on Jurkat cell proliferation. Jurkat cells were grown in the presence or absence of ciprofloxacin as described in the Materials and Methods section. Increase of density of cell suspensions was measured between the 1st and 3rd day as well as between the 8th and 10th day of the experiment. Cells were stained with trypane blue. The cell density at day 3 or 10 is expressed as percentage of the density at day 1 or 8, respectively. Black bars, control cells; grey bars, cells grown in the presence of ciprofloxacin (25 μg/ml). Results represent mean values±S.D. for three independent experiments.

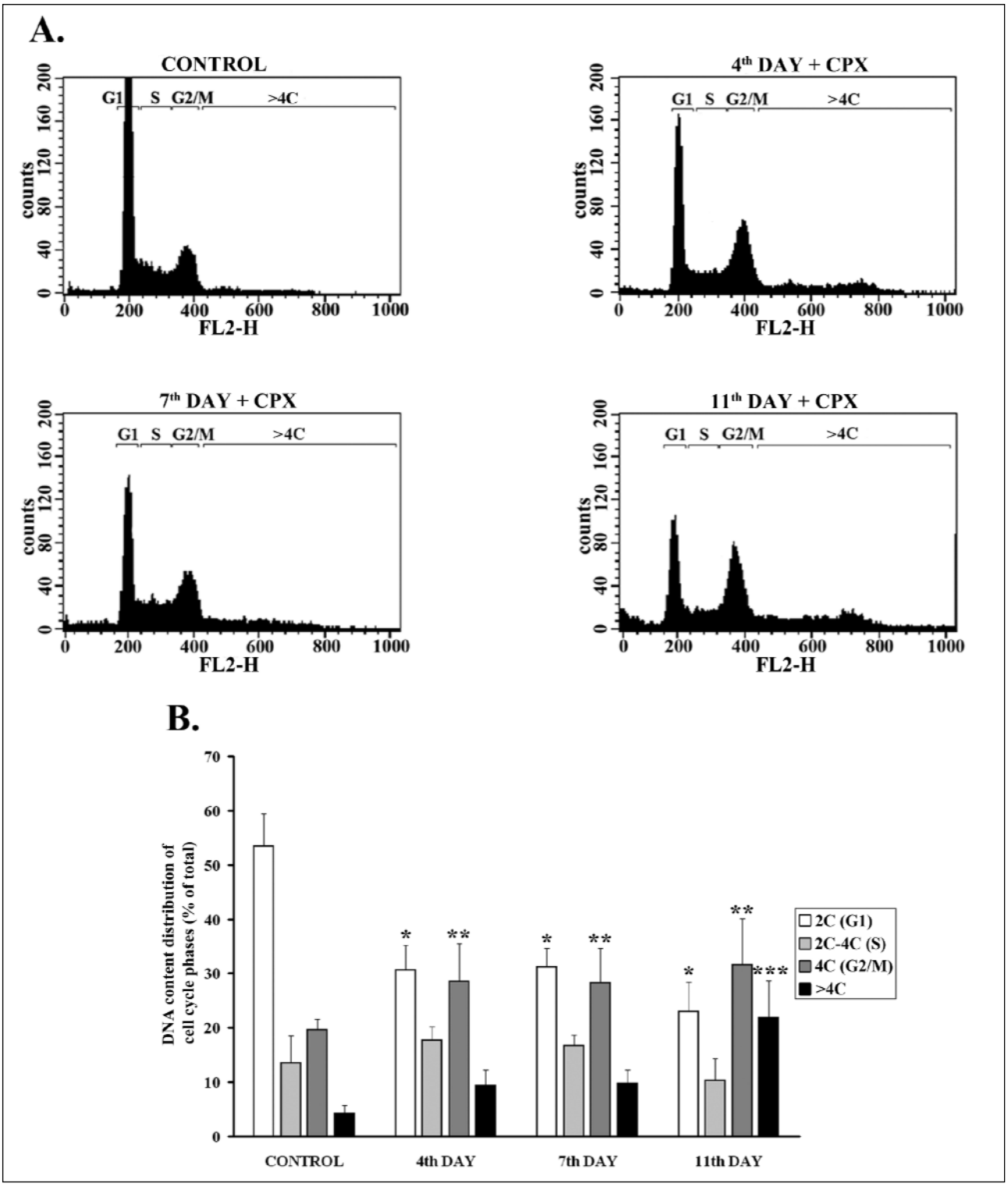

Fig. 2. Effect of ciprofloxacin on the cell cycle. Proportion of cells at various phases of the cell cycle was measured using flow cytometry of fixed cells stained with propidium iodide, as described in the Material and Methods section. Histograms were analyzed using ModFit computer programme. (A) Results of one typical experiment out of three. (B) Combined data from three independent experiments. Results represent means±S.D. White bars, G1 phase; bright-grey bars, S phase; dark-grey bars, G2/M phase; black bars, >4C.

prepared ice-cold solution of acetic acid in methanol (1:3 v/v) was added and the cells were centrifuged for 2 min at 4000x g. The pellet was resuspended in 1 ml of the methanol-acetic acid solution and centrifuged as above. Then, supernatant was drained and the pellet was resuspended in 0.05-0.1 ml of the residual supernatant. Such fixed cells were stored at -20°C.

For chromosome staining, fixed cells were suspended in 0.2 ml of the methanol-acetic acid mixture, and 15 µl samples were dropped on a cover slip sloped at an angle of 45°. After drying at room temperature the preparations were stained for 5-7 min with Karyo® Giemza (Invitrogen) solution, rinsed with water and air-dried.

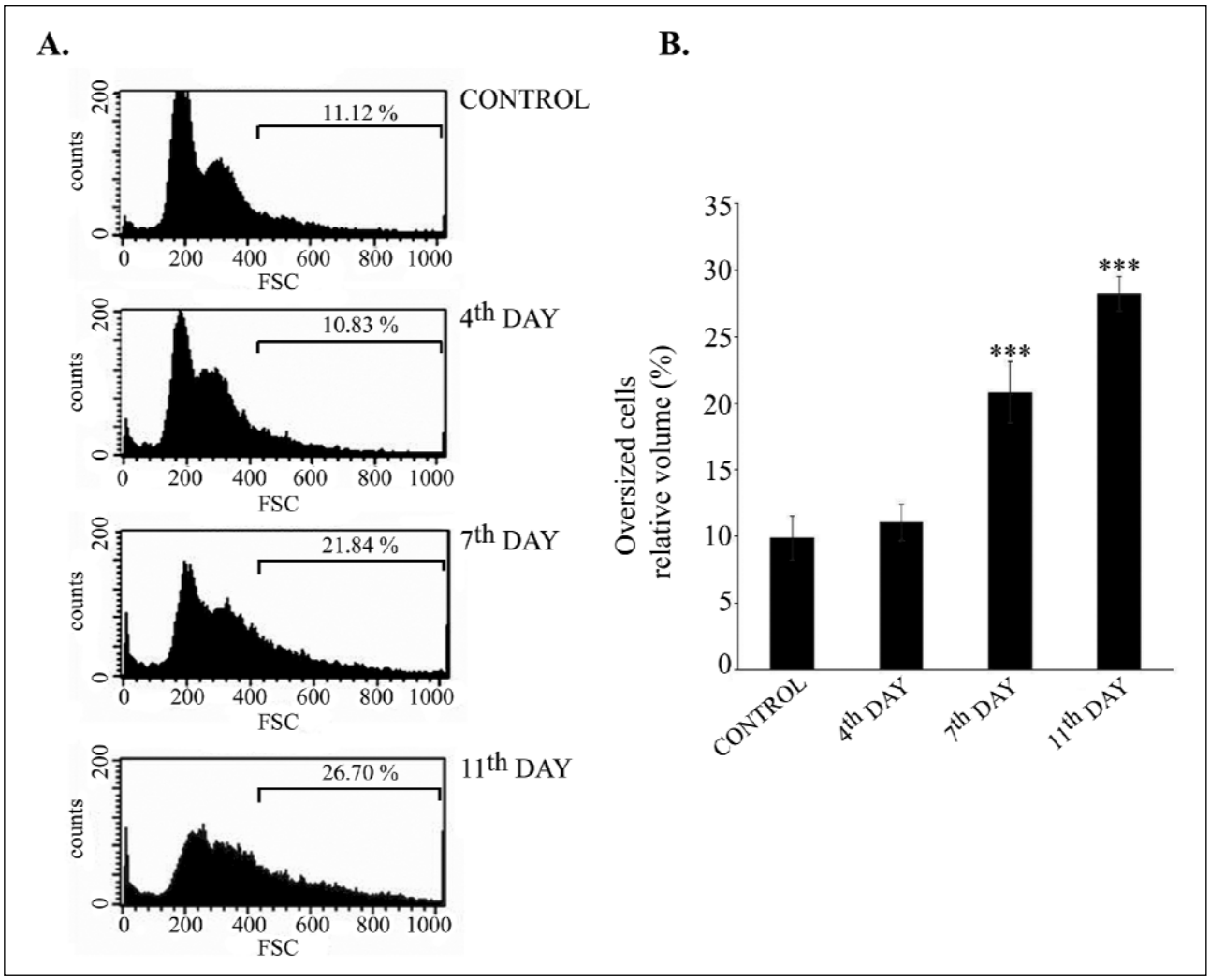

Fig. 3. Effect of ciprofloxacin on cellular size. Jurkat cells were grown in the presence of ciprofloxacin for 7 days and split every two days as described in the Material and Methods section. (A) Histograms showing distribution of cells discriminated on the basis of their relative size. Results of one typical flow cytometry analysis out of three. (B) Combined data from three independent experiments. Results represent means±S.D.

Expression of results

Values shown are means±S.D. for the indicated number of independent experiments. Statistical significance was assessed by Student t-test and expressed as follows: * $p<0.05$, ** $p<0.01$, *** $p<0.001$.

RESULTS

As shown in *Fig. 1* ciprofloxacin progressively slowed cell proliferation, in agreement with earlier observation (4, 14, 15).

Cell cycle analysis (*Fig. 2*) showed that ciprofloxacin applied for 4-11 days did not induce oligonucleosomal DNA fragmentation (lack of "sub-G1" fraction), confirming our previous observation that under conditions used ciprofloxacin does not induce cell death (4). The ratio of cells in the G2/M phase to those in G1 was significantly increased, indicating cell cycle arrest after DNA replication but prior to or during mitosis. This effect was even more conspicuous in cells exposed to 100 μg/ml ciprofloxacin. However, under such conditions oligonucleosomal DNA fragmentation (sub-G1 fraction) was observed, strongly suggesting induction of apoptosis (data not shown).

Fig 2 also shows that cells grown in the presence of ciprofloxacin exhibited a progressive, time-dependent increase of proportion of those with a polydispersed DNA content exceeding 4C (about 23% of cells cultured with ciprofloxacin for 11 days *vs*. 5% in the control cells). This phenomenon indicated polyploidization and aneuploidization. The increase of the DNA content above 4C was accompanied by an increased proportion of oversized cells relative to the population of control cells (*Fig. 3A* and *B*). This additionally supported the thesis that ciprofloxacin did not block the first steps of the cell cycle leading to duplication of the genetic material, but inhibited cell division. To seek the mechanism of this inhibition, organization of the tubulin cytoskeleton was investigated. As shown in *Fig. 4*, some of the ciprofloxacin-treated cells had aberrant mitotic spindles, which was very rare under control conditions. As a consequence of the spindle malformation, an improper multipolar chromosome segregation occurred leading to aneuploidy of cells grown with ciprofloxacin. This conclusion is supported by karyotype analysis which revealed a ciprofloxacin-induced increase of the proportion of cells with aberrant (increased or reduced) number of chromosomes (*Fig. 5*).

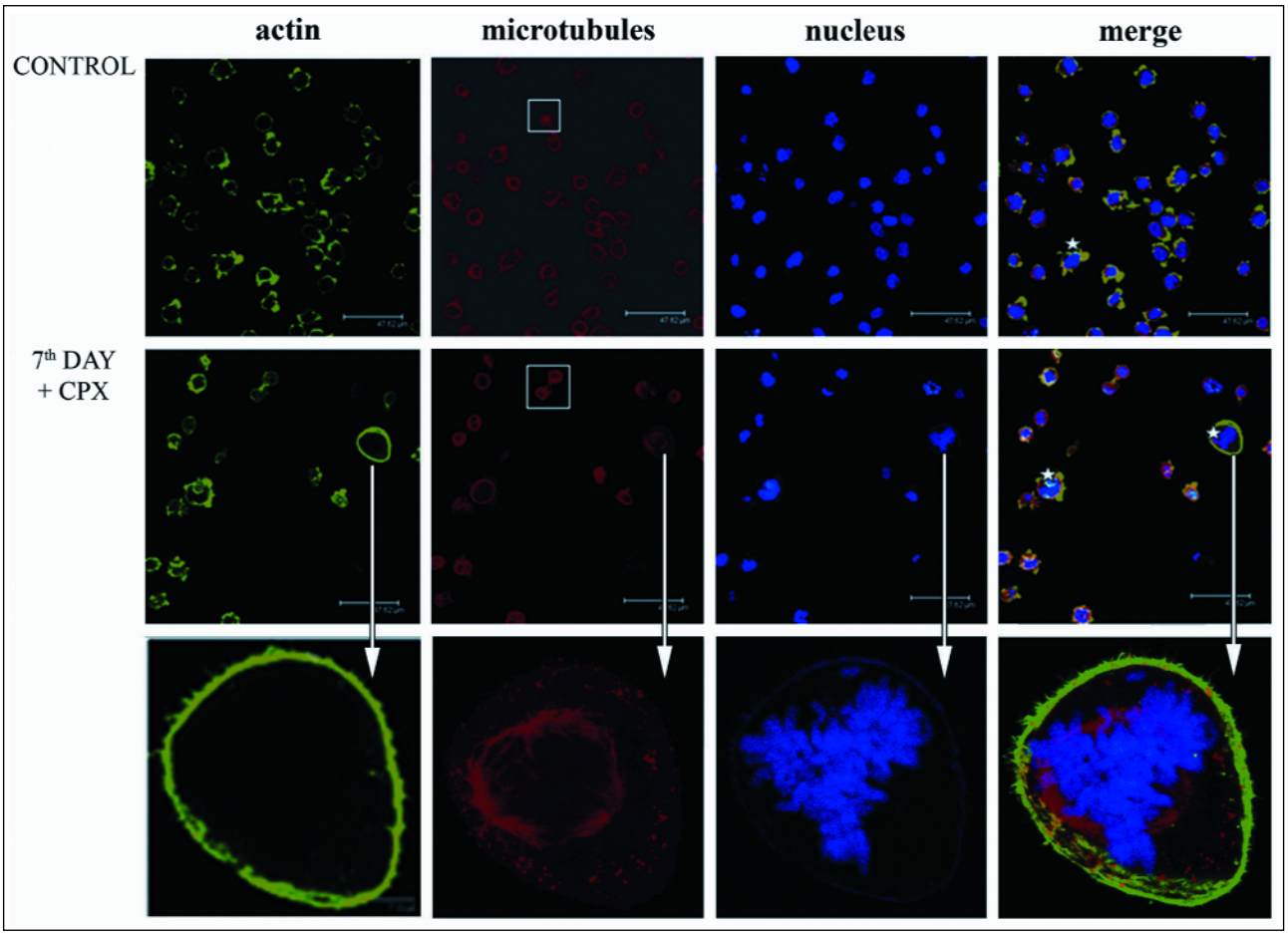

Fig. 4. Ciprofloxacin disturbs mitotic spindle formation. An increased proportion of giant cells is also evident (arrows). The picture shows control cells and cells exposed for 7 days to ciprofloxacin. Green staining, actin cytoskeleton (falloidine coupled with FITC); red staining, α-tubulin (TRITC coupled with secondary antibodies); blue staining, nuclei (DAPI). Note multipolar mitotic spindle in cells grown in the presence of ciprofloxacin. Bar corresponds to 47 μm. Cells were analyzed using confocal microscopy with an oil immersion objective x 63.

DISCUSSION

An antiproliferative action of ciprofloxacin has been described for several cell types to date, and the data presented here conform to the general conclusions from other laboratories, although some differences in experimental protocols and also some exceptions should be noticed. For example, the majority of published data concerning human lymphocytes refer to stimulated cells (16), while in experiments presented in this paper Jurkat cells were not stimulated by any extracellular ligands. Moreover, in opposition to most reports, Gollapudi *et al*. (17) found no effect of this drug (applied at concentrations ranging from 5 to 125 μg/ml) on proliferation of mitogen-stimulated human peripheral blood mononuclear cells or murine splenocytes. It is also noteworthy that Jurkat cells grown in the presence of 25 μg/ml ciprofloxacin did not undergo apoptotic or necrotic death (comprehensive data for this conclusion are not presented here, although the lack of the "sub-G_1" fraction in ciprofloxacin-treated cells, as shown in *Fig. 2*, supports this statement). In contrast, the same cells pretreated with anti-CD3 antibody became susceptible to ciprofloxacin-induced apoptosis, even if this drug was applied at a concentration as low as 2.5 μg/ml (18). Moreover, as it was mentioned above, ciprofloxacin used at concentrations exceeding 50-80 μg/ml induced apoptotic death in many cell types.

We also found that Jurkat cells grown in the presence of 25 μg/ml ciprofloxacin exhibited enhanced IL-2 synthesis, NFAT dephosphorylation and DNA synthesis (detected by BrdUrd incorporation assay) which suggested stimulation of first steps of the cell cycle. Since those observations generally confirm data previously published by other authors (16, 19-20) they are not shown in this paper. However, it should be noted that those changes coincided with an impairment of mitochondrial energy metabolism and store-operated calcium entry (4). Similarly, a substantially increased mean cytosolic [Ca^{2+}] was observed in unstimulated (resting) cells grown in the presence of ciprofloxacin (Koziel, unpublished data). On the other hand, Zehavi-Willner and Shalit found that ciprofloxacin inhibited radioactive thymidine incorporation in Jurkat cells and enhanced IL-2 synthesis in cells stimulated by phytohemagglutinin but not in unstimulated ones (22). However, despite some discrepancies between the data coming from various laboratories, which may result from different experimental conditions, one could draw a general conclusion that ciprofloxacin induces two apparently opposite processes. First, it has an immunomodulatory effect on lymphocytes (enhanced IL-2 synthesis) (23) and second, it inhibits cell proliferation.

Ciprofloxacin-mediated G2/M cell cycle arrest was described previously for rat tendon cells (15). However, in contrast to our data it was not accompanied by aneuploidy formation despite the relatively high concentration of ciprofloxacin used (50 μg/ml). The mechanism of ciprofloxacin-evoked chromosomal instability remains unresolved. It is not clear whether aneuploidy is secondary to the arrested cell

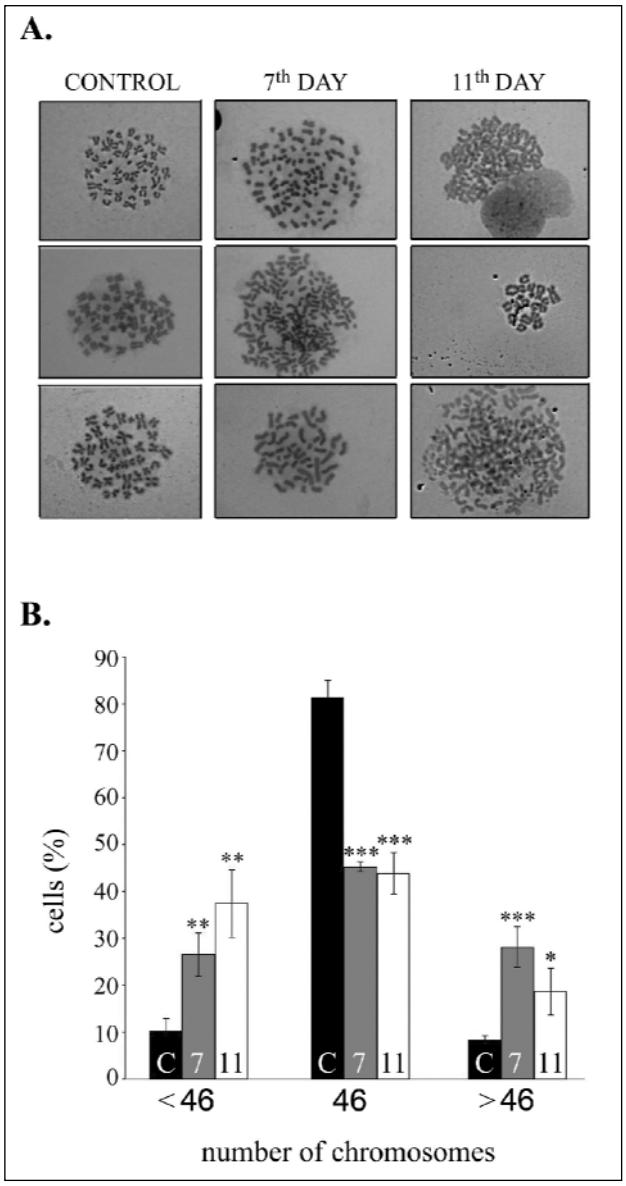

Fig. 5. Effect of ciprofloxacin on karyotype. (A) Typical karyotypes of control and ciprofloxacin-treated cells. An increased as well as decreased number of chromosomes is observed in cells treated with ciprofloxacin for 7 or 11 days. (B) Combined data from three independent experiments. Bars in the middle represent percentage of cells with normal number of chromosomes (46), Bars on the left represent cells with missing chromosomes, represent cells with supernumerary chromosomes. Black, control; grey, the 7th day with ciprofloxacin; white, the 11th day with ciprofloxacin. Results represent mean±S.D.

division or whether impaired cell division results from aberrant karyokinesis.

The data presented in this paper together with those of other authors document the manifold dose-dependent effects of ciprofloxacin on cell physiology. At a concentration of the drug in the growth medium of 15 μg/ml (significantly higher than that found in serum of patients undergoing antimicrobial therapy) no changes in mitochondrial energy metabolism or cell proliferation were noticed for at least 3 weeks of such treatment (Koziel, unpublished observation). On the other hand, if the a concentration of ciprofloxacin exceeded 50 μg/ml, the proportion of apoptotic cells significantly increased already after a few hours of the treatment (Koziel, unpublished data). This observation is in good agreement with data published by other authors, but is beyond the scope of this article (6, 14). In this paper as well as in our previous one we clearly show that 25 μg/ml ciprofloxacin applied for 4-11 days compromises the cell cycle and leads to aneuploidy without activation of apoptosis (4). These changes resemble to some extend effects of low doses of doxorubicin on HCT116 cells (24). It can not be excluded that ciprofloxacin-induced dysfunction of mitochondria (4) together with aberrant chromosome content but without any symptoms of apoptosis also induces stress-induced cellular senescence (25). However, this tempting hypothesis is very preliminary and needs further extensive studies. In Jurkat cells inhibition of cell proliferation occurs despite the "pro-replicative" stimulation of the early events of cell cycle by ciprofloxacin, as described elsewhere (19, 21, 26).

The data showing inhibition of cell growth and formation of aneuploidy without induction of apoptosis may be important when one considers ciprofloxacin as a prospective anticancer drug. Unequal tissue penetration of ciprofloxacin (5) may result in local accumulation of this drug at a concentration which may be insufficient for stimulation of cell death but sufficient for aneuploidy formation. Such ciprofloxacin-induced genomic instability of cells may, at least hypothetically, increase a risk of cancer development and progression (24, 27). On the other hand, it was suggested that the effect of genomic instability on tumorigenesis depends on the level of this disturbance. While a high level of aneuploidy increases probability of cancer development and/or progression, moderate genetic instability may act as a tumour suppressor (27) These effects seem to be dependent on a broad cellular context as well as cell type and origin therefore they are difficult to be precisely predicted. Moreover the growth of cancer cells *in vivo* is influenced by other co-existing cells like macrophages modulating their microenvironment (28). Thus the effect of ciprofloxacin on cancer progression may be more complex.

Abbreviations: BrdUrd: bromodeoxyuridine; DAPI: 4',6-diamino-2-fenylindol; DMSO: dimethyl sulfoxide; FBS: foetal bovine serum; FITC: fluorescein isothiocyanate; PE: phycoerythrin; TRITC: tetramethyl rhodamine isothiocyanate

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REFERENCES

1. Castora FJ, Vissering FF, Simpson MV. The effects of bacterial DNA gyrase inhibitors on DNA synthesis in mammalian mitochondria. *Biochim Biophys Acta* 1983; 740: 417-427.
2. Lawrence JW, Darkin-Rattray S, Xie F, Neims AH, Rowe TC. 4-Quinolones cause a selective loss of mitochondrial DNA from mouse L1210 leukemia cells. *J Cell Biochem* 1993; 51: 165-174.
3. Lawrence JW, Claire DC, Weissig V, Rowe TC. Delayed cytotoxicity and cleavage of mitochondrial DNA in ciprofloxacin-treated mammalian cells. *Mol Pharm* 1996; 55: 178-188.
4. Koziel R, Zablocki K, Duszynski J. Calcium signals are affected by ciprofloxacin as a consequence of reduction of mitochondrial DNA content in Jurkat cells. *Antimicrob Agents Chemother* 2006; 50: 1664-1671.

5. Gerding DN, Hitt JA. Tissue penetration of the new quinolones in humans. *Rev Infect Dis* 1989; 11(Suppl 5): S1046-S1057.
6. Smart DJ, Halicka HD, Traganos F, Darzynkiewicz Z, Williams GM. Ciprofloxacin-induced G2 arrest and apoptosis in TH6 lymphoblastoid cells is not dependent on DNA double-strand break formation. *Cancer Biol Ther* 2008; 7: 113-119.
7. Aranha O, Grignon R, Fernandes N, McDonnel T, Wood DP Jr, Sarkar FH. Suppresion of human prostate cancer cell growth by ciprofloxacin is associated with cell cycle arrest and apoptosis. *Int J Oncol* 2002; 22: 787-794.
8. Aranha O, Zhu L, Alhasan S, Wood DP Jr, Kuo TH, Sarkar FH. Role of mitochondria in ciprofloxacin induced apoptosis in bladder cancer cells. *J Urol* 2002; 167: 1288-1294.
9. Herold C, Ocker M, Ganslmayer M, Gerauer H, Hahn EG, Schuppan D. Ciprofloxacin induces apoptosis and inhibits proliferation of human colorectal carcinoma cells. *Br J Cancer* 2002; 86: 443-448.
10. Miclau T, Edin ML, Lester GE, Lindsey RW, Dahners LE. Effect of ciprofloxacin on the proliferation of osteoblast-like MG-63 human osteosarcoma cells in vitro. *J Orthop Res* 1998; 16: 509-512.
11. Somekh E, Douer D, Shaked N, Rubinstein E. In vitro effects of ciprofloxacin and pefloxacin on growth of normal human hematopoietic progenitor cells and on leukemic cell lines. *J Pharmacol Exp Ther* 1989; 248: 415-418.
12. Aranha O, Wood DP Jr, Sarkar FH. Ciprofloxacin mediated cell growth inhibition, S/G2-M cell cycle arrest, and apoptosis in a human transitional cell carcinoma of the bladder cell line. *Clin Cancer Res* 2000; 6: 891-900.
13. Makowska A, Zablocki K, Duszynski J. The role of mitochondria in the regulation of calcium influx into Jurkat cells. *Eur J Biochem* 2000; 267: 877-884.
14. Pessina A, Bonomi A, Casati S, *et al*. Mitochondrial function, apoptosis and cell cycle delay in the WEHI-3B leukaemia cell line and its variant ciprofloxacin-resistant WEHI-3B/CPX. *Cell Prolif* 2007; 40: 568-579.
15. Tsai W-C, Hsu C-C, Tang F-T, Wong AMK, Chen Y-C, Pang J-HS. Ciprofloxacin-mediated cell proliferation inhibition and G2/M cell cycle arrest in rat tendon cells. *Arthritis Rheum* 2008; 58: 1657-1663.
16. Forsgren A, Bredberg A, Riesbeck K. Effects of ciprofloxacin on human lymphocytes – laboratory studies. *Scand J Infect Dis* 1989; Suppl 60: 39-45.
17. Gollapudi SV, Prabhala RH, Thadepalli H. Effect of ciprofloxacin on mitogen-stimulated lymphocyte proliferation. *Antimicrob Agent Chemother* 1986; 29: 337-338.
18. Jun Y-T, Kim H-J, Song M-J, *et al.* In vitro effects of ciprofloxacin and roxithromycin on apoptosis of Jurkat T lymphocytes. *Animicrob Agent Chemother* 2003; 47: 1161-1164.
19. Forsgren A, Bergh A-K, Brandt M, Hansson G. Quinolones affect thymidine incorporation into the DNA of human lymphocytes. *Antimicrob Agents Chemother* 1986; 29: 506-508.
20. Forsgren A, Bredberg A, Riesbeck K. New quinolones: in vitro effects as a potential source of clinical toxicity. *Rev Infect Dis* 1989; 11(Suppl 5): S1382-S1388.
21. Riesbeck K, Forsgren A. Increased interleukin 2 transcription in murine lymphocytes by ciprofloxacin. *Immunopharmacology* 1994; 27: 155-164.
22. Zehavi-Willner T, Shalit I. Enhancement of interleukin-2 production in human lymphocytes by two new quinolone derevatives. *Lymphokine Res* 1989; 8: 35-45.
23. Riebeck K, Forsgren A, Henriksson A, Bredberg A. Ciprofloxacin induces an immunomodulatory stress response in human T lymphocytes. *Antimicrob Agents Chemother* 1998; 42: 1923-1930.
24. Sliwinska MA, Mosieniak G, Wolanin K, *et al*. Induction of senescence with doxorubicin leeds to increased genomic instability of HCT116 cells. *Mech Ageing Dev* 2008; 130: 24-32.
25. Kaszubowska L. Telomere shortening and ageing of the immune system. *J Physiol Pharmacol* 2008; 59(Suppl 9): 169-186.
26. Riesbeck K, Andersson J, Gullberg M, Forsgren A. Fluorinated 4-quinolones induce hypeproduction of interleukin 2. *Proc Natl Acad Sci USA* 1989; 86: 2809-2813.
27. Weaver BAA, Cleveland DW. Aneuploidy: instigator and inhibitor of tumorigenesis. *Cancer Res* 2007; 67: 10103-10105.
28. Kaczmarek M, Frydrychowicz M, Nowicka A, *et al*. Influence of pleural macrophages on proliferative activity and apoptosis regulating proteins of malignant cells. *J Physiol Pharmacol* 2008; 59(Suppl 6): 321-330.

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Author's address: Prof. Krzysztof Zablocki, Nencki Institute of Experimental Biology, 3 Pasteura Street, 02-093 Warsaw, Poland; Phone: (+4822) 5892146; Fax (+4822) 8225342; E-mail: k.zablocki@nencki.gov.pl