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Ciprofloxacin Does Not Inhibit Mitochondrial Functions but Other Antibiotics Do

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At clinical concentrations, ciprofloxacin did not inhibit mitochondrial DNA replication, oxidative phosphorylation, protein synthesis, or mitochondrial mass (transmembrane potential). No difference in supercoiled forms of DNA was observed. The tetracyclines and chloramphenicol inhibited protein synthesis at clinically achievable concentrations, while rifampin, fusidic acid, and clindamycin did not.

The effects of 4-quinolones on mammalian cells remain obscure. Calf thymus DNA polymerase α primase complex and topoisomerases I and II are inhibited by the drugs but not at clinically achievable concentrations (17, 21). The 4-quinolones at therapeutic concentrations lead to an increase of thymidine incorporation in phytohemagglutinin-stimulated lymphocytes (11, 12). Furthermore, the 4-quinolones inhibit cell cycle progression (13), mitogen-induced mononuclear cell proliferation (15), and immunoglobulin secretion (13). The synthesis of interleukin-1 is inhibited at high concentrations (26), while the production of interleukin-2 is up-regulated (23, 25, 27) at the mRNA level (25). DNA strand break induction by ciprofloxacin has also been reported (3).

Mitochondria resemble bacteria in numerous ways (20), and the ancestry of the mitochondrial (mt) rRNA genes of all eucaryotes has been traced to the eubacteria (16). Topoisomerases I and II have been discovered in mammalian mitochondria (4, 9), but an mt gyrase (a type II topoisomerase) has not been detected. In bacteria, the 4-quinolones inhibit the enzyme DNA gyrase (1, 35) through direct binding to the enzyme or through the formation of a drug-DNA complex (29, 30, 33). The bacterial gyrase inhibitor ciprofloxacin penetrates human cells effectively (34) and may therefore hypothetically act upon mitochondria.

Fresh solutions of the following preservative-free drugs were used: benzylpenicillin (ACO Pharmaceuticals, Solna, Sweden), chloramphenicol (Sigma Chemical Co., St. Louis, Mo.), ciprofloxacin (Bayer, Wuppertal, Federal Republic of Germany), clindamycin hydrochloride (The Upjohn Co., Brussels, Belgium), doxycycline hydrochloride (Pfizer, Brussels, Belgium), sodium fucidate (Leo Pharmaceutical Products, Ballerup, Denmark), rifampin (MDRI-Lepetit Research Center, Gerenzano, Italy) and tetracycline chloride (ACO). Rifampin was solubilized in methanol to 50 mg/ml, and chloramphenicol was solubilized in ethanol to the same concentration.

Liver mitochondria were isolated from Wistar rats (200 to 300 g) (ALAB, Sollentuna, Sweden), with the omission of perfusion, on a Percoll gradient (Pharmacia, Uppsala, Sweden) (24). ADP, dithiothreitol, and sodium succinate were omitted from the buffers. For preparation of a lysate (31), mitochondria were isolated in a 0.25 M sucrose buffer by differential centrifugation and treated with 0.025 μg of digitonin (Merck & Co., Inc., Rahway, N.J.) per mg of mt protein (protein analysis method; Bio-Rad Laboratories, Richmond, Calif.) for 10 min.

Protein synthesis in isolated mitochondria (19) was studied by measuring [^{3}H]leucine incorporation (40 μCi/ml; NET-135H; specific activity, 60 Ci/mmol; New England Nuclear Corp., Boston, Mass.). Mitochondria (3 to 10 mg of protein per ml) were preincubated with antibiotics for 15 min and incubated for 45 min with [^{3}H]leucine. In all experiments, each antibiotic concentration was tested in triplicate.

DNA replication experiments were performed as described above (19), but with some adjustments. The following were added: 10 mM $MgCl_2$, 2.5 mM phosphoenolpyruvate (Sigma), and a 0.32 mM concentration of each of the unlabeled nucleotides. Mitochondria (5 to 15 mg of mt protein per ml) were preincubated with antibiotics and, after the addition of either [^{3}H]dTTP (45 μCi/ml; NET-221A; specific activity, 81.7 Ci/mmol; New England Nuclear) or [^{32}P]dATP (80 μCi/ml; NEG-012Z; specific activity, 5,000 Ci/mmol; New England Nuclear), were incubated for 60 min at 37°C. Actinomycin D or C1 (Sigma) was used as the control substance inhibiting DNA replication (22). The same concentrations of nucleotides as those mentioned above were used in mt lysate DNA replication (31). No preincubation with ciprofloxacin was performed for the lysate. mt protein and mt DNA were precipitated on ice with trichloroacetic acid. mt DNA, isolated by conventional methods, was run on a 0.7% agarose gel either immediately or after incubation with *Eco*RI, *Hin*dIII, or *Xba*I (Boehringer GmbH, Mannheim, Federal Republic of Germany) or *Ava*I (Toyobo International, Osaka, Japan). Southern blotting was performed, and the membrane was exposed to X-ray film at −70°C.

Oxidative phosphorylation was measured in a volume of 0.65 ml (28), and 5 mM pyruvate plus 5 mM L-malate or 5 mM L-glutamate plus 2.5 mM L-malate served as fuel. Mitochondria and antibiotics were incubated for 10 to 15 min, and 0.2 mM ADP was added. Four different experiments were performed at every concentration. Benzylpenicillin was used as the control substance not affecting phosphorylation.

Human lymphoblastoid Raji cells were grown for 24 h in RPMI 1640 medium (GIBCO Bio-Cult, Ltd., Edinburgh, Scotland) in the presence of antibiotics (11). Before being run in a flow cytometer (FacScan; Becton Dickinson, Mountain View, Calif.), 5×10^6 Raji cells were incubated with 10 μg of rhodamine 123 (Kodak, Jakobsberg, Sweden) per ml (8) for 10 min at 37°C and subsequently washed with RPMI 1640. The specific uptake was verified with 10 mM dinitrophenol (Sigma) (6). The cells were excitated at 488 nm, green

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TABLE 1. DNA replication in whole mitochondria and an mt lysate

Sample and drug	Incorporation of [^{3}H]dTTP at a drug concn of[a]:		
	100 μg/ml	20 μg/ml	5 μg/ml
Whole mitochondria			
Ciprofloxacin	90[b] ± 4.1 (15)	97 ± 3.0 (15)	100 ± 5.9 (15)
Actinomycin C1	13[b] ± 2.0 (4)	NT[c]	NT
mt lysate			
Ciprofloxacin	91 ± 6.7 (8)	88 ± 11 (7)	110 ± 10 (7)
Actinomycin C1	5.8[d] ± 1.4 (4)	NT	NT

[a] Expressed as the mean percentage of the control value± the standard error of the mean. Numbers of experiments are shown in parentheses.
[b] $P < 0.01$.
[c] NT, Not tested.
[d] $P < 0.001$.

fluorescence was measured at 525 nm, and 10,000 events were registered from every sample. In all experiments, Student's t test for paired data was used to calculate the statistical significance of differences.

When isolated mitochondria were incubated with ciprofloxacin in vitro, the incorporation of [^{3}H]dTTP into mt DNA was slightly reduced (10%) at 100 μg/ml (Table 1). mt DNA replication was linear for 2 h, and ciprofloxacin did not affect the kinetics. In contrast, pronounced inhibition of mt DNA replication was obtained with actinomycin C1. For comparison, the incorporation of thymidine into DNA of *Escherichia coli* is 20% of the control level at the MICs of ciprofloxacin (unpublished results) and nalidixic acid (32). An mt lysate was made to test whether the mt outer and inner membranes act as barriers, but ciprofloxacin had no significant effect on DNA replication in the lysate (Table 1). The incorporation of [^{3}H]dTTP in controls was, on the average, 4,100 cpm for whole mitochondria and 1,300 cpm for the lysate.

To elucidate whether the forms of DNA were affected by ciprofloxacin, we examined DNA from mitochondria which had been exposed to ciprofloxacin and incubated for 60 min with [^{32}P]dATP. No visible differences in nicked-relaxed circular or supercoiled DNA in mitochondria exposed to ciprofloxacin could be seen. Neither was the distinction of ^{32}P activity among restriction enzyme fragments affected, indicating that the label was incorporated evenly among early and late replicating parts of the mt DNA. In the search for an mt DNA gyrase, Castora et al. (5) found that the incorporation of nucleotides into mt DNA decreased when mitochondria were exposed to nalidixic acid (100 to 300 μg/ml) and novobiocin (50 to 500 μg/ml). However, in the same laboratory (14), it was recently reported that these drugs, at the high concentrations used, had a "nonspecific toxic" effect by inhibiting oxidative phosphorylation. When we measured oxidative phosphorylation, it was not at all affected by ciprofloxacin at clinical concentrations, but at 100 μg/ml ciprofloxacin significantly inhibited the respiratory control index by 47% ($P < 0.01$) and 71% ($P < 0.001$) with pyruvate and L-glutamate, respectively, as substrates.

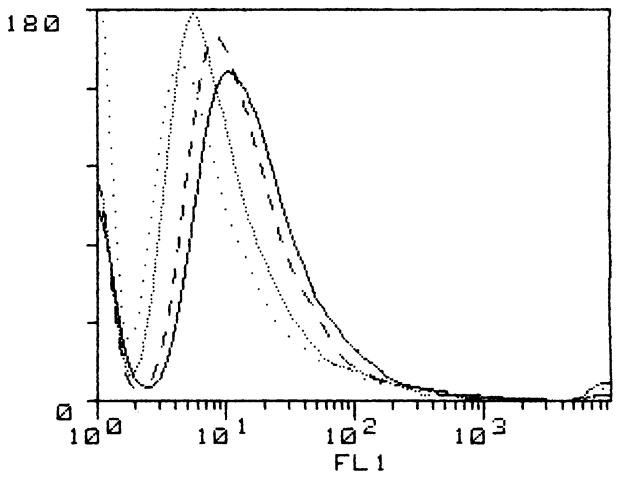

FIG. 1. Inhibition of the uptake of rhodamine 123 by doxycycline at 10 (. . . .), 5 (.....), and 1.25 (– – – –) μg/ml as compared with uptake in Raji cells cultured for 24 h in the absence of antibiotics (——). The cells were run in a flow cytometer. FL 1 (fluorescence 1) expresses the fluorescence of rhodamine 123 at 525 nm. The y axis represents the relative number of cells.

Ciprofloxacin and the bacterial protein synthesis inhibitor clindamycin had no effects on the incorporation of [^{3}H]leucine (on the average, 7,100 cpm in the controls) into mt protein (Table 2). Clindamycin penetrates human cells effectively but does not inhibit lymphocyte functions at concentrations from 1.0 to 25 μg/ml (10), consistent with previous findings that mitochondria are impermeable to macrolide antibiotics (18). Fusidic acid and rifampin significantly inhibited protein synthesis only at the highest concentrations tested. At a concentration of 1.25 μg/ml, doxycycline inhibited [^{3}H]leucine incorporation by 60%. Tetracycline and chloramphenicol inhibited protein synthesis by approximately 30% at 5 μg/ml (Table 2). These results are consistent with studies in our and other laboratories demonstrating an immunosuppressive effect of doxycycline both in vitro and in animal experiments (2, 10). These antibiotics act in mitochondria in a similar fashion as in bacteria (7, 18).

TABLE 2. mt protein synthesis in the presence of some antibiotics

Drug	Incorporation of [^{3}H]leucine at a drug concn of[a]:				
	100 μg/ml	20 μg/ml	5 μg/ml	1.25 μg/ml	0.31 μg/ml
Ciprofloxacin	84 ± 7.9 (5)	87 ± 7.1 (5)	87 ± 1.4 (4)		
Clindamycin	90 ± 3.4 (3)	115 ± 16 (3)	80 ± 11 (2)		
Fusidic acid	67[b] ± 8.4 (3)	84 ± 13 (3)	84 ± 6.9 (3)		
Rifampin	42[b] ± 6.5 (2)	91 ± 14 (3)	96 ± 4.3 (3)		
Chloramphenicol			65[b] ± 6.6 (3)	70 ± 11 (3)	90 ± 3.2 (3)
Tetracycline			68[b] ± 3.7 (3)	87 ± 4.7 (3)	88[b] ± 2.0 (3)
Doxycyline			12[c] ± 3.1 (3)	40[c] ± 5.8 (3)	71 ± 5.0 (3)

[a] See Table 1, footnote *a*.
[b] $P < 0.05$.
[c] $P < 0.01$.

To study intracellular mitochondria, we performed staining with rhodamine 123, a positively charged fluorochrome. Rhodamine 123 stains mitochondria selectively, is dependent on the electrochemical potential across the mt inner membrane, and also reflects the mt mass. When fluorescence was subsequently measured, no difference between cells treated with ciprofloxacin (5 and 20 μg/ml) and control cells without antibiotics were detected when Raji cells were incubated with ciprofloxacin for 24 h. However, doxycycline inhibited the uptake of rhodamine 123 (Fig. 1).

In conclusion, of many antibiotics tested, only chloramphenicol and the tetracyclines (particularly doxycycline) inhibited mitochondria at clinically achievable concentrations. However, the 4-quinolone ciprofloxacin had no apparent effect on mitochondria. Thus, an inhibited mt function is not likely to be accounted for by ciprofloxacin-altered lymphocyte functions (12, 13).

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