Fluoroquinolones Protect the Human Lymphocyte CEM Cell Line from HIV-1-Mediated Cytotoxicity

Junko Nozaki-Renard¹, Takashi Iino², Yoshinari Sato², Yasumasa Marumoto², Genkichi Ohta² and Mitsuru Furusawa^{2†}

¹Department of Microbiology, Tokyo Medical College, Shinjuku 6-chome, Shinjuku-ku, Tokyo 160 and

²Molecular Biology Research Laboratory, Research Institute, Daiichi Pharmaceutical Co., Ltd., 16–13, Kitakasai 1-chome, Edogawa-ku, Tokyo 134, Japan

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ABSTRACT. Infection of the human lymphocyte CEM cell line with the HIV-1 (human immunodeficiency virus type-1, LAV-1 strain) results in cell death. A fluoroquinolone antibiotic, ofloxacin, protected the infected cells from HIV-1-mediated cytolysis. Other fluoroquinolones, e.g. ciprofloxacin, norfloxacin, and enoxacin, also protected the infected cells from HIV-1-mediated cytolysis. The d-isomer of ofloxacin (DR-3354) was about 50-fold less effective than the l-isomer (DR-3355). Almost none of the rescued cells had detectable HIV-antigens and they could be maintained for long periods *in vitro* without drugs.

The new fluoroquinolones, such as ofloxacin (17), ciprofloxacin (24), and norfloxacin (10), are chemical compounds derived from nalidixic acid and possess potent antibacterial activity (14). They have been used clinically for a variety of infectious diseases due to their broad spectrum of target bacteria and relatively weak side effects (9). Although there have been several reports describing inhibitory activity of quinolone related compounds against reverse transcriptase of murine and avian retroviruses (20) and HIV-1 (Fifth Int. Conf. AIDS Abstract: # C624), no studies have been reported for HIV-1-mediated cytotoxicity. These viruses have gained particular importance recently due to the finding that HIV-1 mediates the onset of the acquired immunodeficiency syndrome (AIDS) (1, 13).

AIDS is characterized by a progressive depletion of the helper T-lymphocyte subset (11, 25). HIV-1 infection of susceptible human lymphocyte cell lines and subsequent cell destruction constitute a valid *in vitro* model system for the action of HIV in generating AIDS. HIV-1 infection of these cell lines inevitably leads to cell death and thus they provide a useful screening system for potential anti-AIDS drugs (6). In this report, we demonstrate that several fluoroquinolones protect cells from HIV-mediated cytotoxicity.

MATERIALS AND METHODS

Infection and culture of cells. CEM cells (16) were used to

test the action of the various fluoroquinolones on HIV-1 (LAV-1 strain, a gift from Dr. L. Montagnier) infected cells. Cell-free supernatants from virus-infected CEM cells were used as the virus source. The supernatants contained approximately 5×10^6 TCID₅₀/ml (TCID₅₀ = 50% tissue culture infectious dose) and were stored at -70°C before use.

CEM cells were cultured in RPMI 1640 supplemented with 10% FCS in humidified 5% CO₂/air at 37°C . The cells were infected with HIV-1 on day 0 by adding the stock viral solution to the host cells at MOI (multiplicity of infection) = 0.05 and incubating at 37°C for 90 min with stirring at 30 min intervals. Fresh culture medium was added to the infected cells for a cell density of 2.5×10^5 cells/ml to give the HIV-infected cell suspension, and 2 ml of the cell suspension was added to each well of 24-well culture plates (2.5×10^5 cells/ml). Under these experimental conditions, HIV-1-infected cells died within about 3 weeks; however, in some experiments a few cells were rescued spontaneously without any drug. This phenomenon has been already reported by Folks *et al.* (3).

Fluoroquinolone treatment. Drugs were dissolved in physiological saline and added to the cell medium after the viral infection on day 0 at the final concentrations indicated in Figure 1 and Table I. The culture medium was changed on days 4, 10, 15, 20, and 25 by agitating the cultures with a pipette to suspend the cells and adding 0.5 ml of cell suspension to 2 ml of fresh medium without drug. Thus, the fluoroquinolones were initially present at the indicated concentrations for only 4 days post-infection and the compounds were progressively diluted by 1 : 5 with each medium change. On days 4, 7, 10, 20, and 30, the number of viable cells, viability, and number of HIV antigen-positive cells were determined. Cell viability was

† To whom correspondence should be addressed.

determined by trypan blue exclusion.

Immunofluorescence for HIV-1 antigens. The presence of HIV antigen in infected cells was determined by indirect immunofluorescence microscopy using serum from an HIV-1-infected asymptomatic carrier. Cells were mounted on a slide, fixed with acetone for 10 min at -20°C , incubated with 150-fold diluted carrier serum, and stained with 50-fold diluted FITC-conjugated goat anti-human IgG F(ab')₂ fragments (Cappel).

RESULTS

Infection of CEM cells with HIV-1 led to essentially a complete loss of cell viability within 30 days, and by day 4 about 80% of the cell were positive for HIV antigen (Fig. 1, Table I). In contrast, incubation with ofloxacin protected a significant subpopulation of cells from HIV-mediated cytotoxicity (Fig. 1). The rate of appearance of the surviving cells was dose-dependent between 1 and 10 $\mu\text{g}/\text{ml}$ (Fig. 1a), and 5 $\mu\text{g}/\text{ml}$ ofloxacin was sufficient for 90% viability by day 10 (Fig. 1b). Maintenance of cell viability was paralleled by a gradual loss of HIV antigen-positive cells to less than 1% at day 30 (Fig. 1c).

Incubation of the HIV-infected cells with a variety of other fluoroquinolones resulted in surviving cell populations in a manner similar to that observed with ofloxacin (Table I). The rate of appearance of the surviving cells was dose-dependent for each fluoroquinolone tested, and 10 $\mu\text{g}/\text{ml}$ drug was sufficient for more than 90% viability by day 30. Other than ofloxacin, the most effective compounds were ciprofloxacin, DR-3355 (l-isomer of ofloxacin), and the Ia derivative of ofloxacin (7), all of which have comparable antibacterial activity (7, 14, 23). The d-isomer of ofloxacin (DR-3354) was about 50-fold less effective than the l-isomer (DR-3355), which reflects similar differences in their antibacterial activity (8). Despite comparable antibacterial activity (unpublished data), the n-propyl derivative of ofloxacin (Ia) was more effective than the 4-acetyl derivative (Ib). This may be related to their relative degrees of hydrophobicity and the consequently greater ability of the n-propyl compound to penetrate the cell membrane. The quinolone analogue, enoxacin (2), also had cell-protecting activity.

The optimal concentration of ofloxacin and DR-3355 for protection of the infected cells had no effect on cell viability of uninfected control cells when cultured continuously with either drug for up to 32 days (data not shown). However, toxicity was observed after continuous culture with 50 $\mu\text{g}/\text{ml}$ DR-3355 ($5 \times$ optimal concentration) as judged by a decrease in the cell growth rate (data not shown).

Pre-treatment of CEM cells with DR-3355 followed by HIV exposure did not protect the cells from HIV-

Fig. 1. Ofloxacin rescues HIV-1-infected CEM cells. CEM cells were infected with HIV-1 on day 0 and incubated at 37°C from day 0–4 with final concentrations of ofloxacin of 1 $\mu\text{g}/\text{ml}$ (▲), 2.5 $\mu\text{g}/\text{ml}$ (△), 5 $\mu\text{g}/\text{ml}$ (●), or 10 $\mu\text{g}/\text{ml}$ (○). Control cells included infected cells incubated with saline (■) and non-infected cells (□). Drug concentrations were diluted 5-fold at each medium change on days 4, 10, 15, 20, and 25. On the days indicated, cells were tested for viable cell number (a), percent viability (b), and percent HIV antigen-positive cells (c) as described in Materials and Methods.

Table I. EFFECT OF FLUOROQUINOLONES ON HIV-1-INFECTED CEM CELLS.

concentration ($\mu\text{g/ml}$)	control		OFLX		CPFX		NFLX		ENX		DR-3355		DR-3354		Ia		Ib		AZT	
	0	1	10	1	10	1	10	1	10	1	10	1	10	1	10	1	10	1	10	1
day																				
Viable cells ($\times 10^4$)	4	270	200	180	220	180	230	180	220	140	220	170	150	130	220	190	250	230	320	280
	7	40	58	79	86	65	52	60	64	52	71	50	41	38	53	62	44	64	230	220
	10	20	32	370	52	250	26	250	29	150	54	280	19	98	19	240	44	68	200	240
	20	7	2	480	8	300	<1	370	7	180	3	230	3	7	25	320	NT	NT	<1	<1
	30	<1	27	470	33	330	<1	400	<1	380	68	320	<1	240	14	300	NT	NT	<1	<1
Viable cell percentage (%)	4	92	94	93	84	91	94	92	95	97	95	92	94	86	89	89	94	92	98	98
	7	55	62	74	73	77	59	71	66	61	70	68	65	66	75	70	57	63	96	98
	10	31	33	90	53	92	30	91	41	91	61	94	30	75	27	83	56	67	75	91
	20	9	13	99	53	96	<1	97	50	96	20	85	4	41	81	92	NT	NT	<1	<1
	30	<1	93	97	92	95	<1	94	0	94	98	92	<1	96	36	94	NT	NT	<1	<1
Viable antigen- positive cell percentage (%)	4	81	85	88	83	88	88	92	88	88	91	88	90	92	88	80	87	86	4	<1
	7	80	65	68	62	57	75	63	70	68	61	57	67	45	62	50	55	60	73	9
	10	UN	48	37	37	27	UN	5	UN	30	56	31	UN	27	UN	40	UN	40	84	64
	20	UN	43	2	33	10	UN	4	UN	5	NT	19	UN	UN	UN	13	NT	NT	NT	NT
	30	UN	NT	<1	NT	<1	NT	<1	NT	<1	NT	<1	NT	1	UN	4	NT	NT	NT	NT

Abbreviations: UN, Undeterminable due to substantial cell debris; NT, Not tested; OFLX, Ofloxacin; CPFX, Ciprofloxacin; NFLX, Norfloxacin; ENX, Enoxacin; DR-3355; (l-isomer of ofloxacin), 3-(S)-9-Fluoro-2, 3-dihydro-3-methyl-10-(4-methyl-1-piperazinyl)-7-oxo-7H-pyrido [1, 2, 3-de] [1, 4] benzoxazine-6-carboxylic acid; DR-3354: (d-isomer of ofloxacin), 3-(R)-9-Fluoro-2, 3-dihydro-3-methyl-10-(4-methyl-1-piperazinyl)-7-oxo-7H-pyrido [1, 2, 3-de] [1, 4] benzoxazine-6-carboxylic acid; Ia: ($\pm$)-9-Fluoro-2, 3-dihydro-3-methyl-10-(4-n-propyl-1-piperazinyl)-7-oxo-7H-pyrido [1, 2, 3-de] [1, 4] benzoxazine-6-carboxylic acid; Ib: ($\pm$)-10-(4-Acetyl-1-piperazinyl)-9-fluoro-2, 3-dihydro-3-methyl-7-oxo-7H-pyrido [1, 2, 3-de] [1, 4] benzoxazine-6-carboxylic acid.

induced cytotoxicity. Novobiocin, which is a non-quinolone antibacterial drug, showed weak cell-protecting activity at 100 µg/ml, and nalidixic acid had no antiviral effect (data not shown). In control experiments, 3'-azido-3'-deoxythymidine (AZT) dramatically protected CEM cells from HIV-1 cytotoxicity up to day 10 by inhibiting viral propagation (4, 15), but eventually all the cells were killed by the infection by day 20 without additional AZT (Table I).

Interestingly, the surviving CEM cell populations had very few HIV antigen-positive cells as shown in Table I and Figure 1c. Related to this is the observation that protected cells arising from the initial 4-day treatment with ofloxacin or DR-3355 have been maintained in culture for at least 3 months so far in the absence of drugs with no loss in cell viability. Essentially the same results were obtained with HUT-78 and MT-4 cells, except that the surviving populations had a higher percentage of cells with HIV antigen and HUT-78 cells required continuous culture with DR-3355 for survival (data not shown).

DISCUSSION

While the mechanism of HIV-induced cytotoxicity is unresolved (18, 19, 21), the notion that the fluoroquinolones interrupt a critical viral function is supported by the finding that drug treatment of infected cells, but not pre-treatment of control cells, results in protection from HIV-mediated cytotoxicity. The antibacterial activity of the fluoroquinolones is reportedly related to effects on bacterial gyrase (5, 22), and similar effects on functionally related HIV-1 proteins (e.g. integrase) can be hypothesized as a possible mode of action resulting in cell survival. Recently, tetracycline derivatives were reported to protect HIV-1-infected cells against cytolysis. The drug target was speculated to be mycoplasma (12), although there was neither evidence presented for the presence of mycoplasma nor for mycoplasma involvement in the cytopathology characteristic of HIV-1 infected cells. Regardless of the mechanism, it is clear that fluoroquinolone treatment of HIV-1 infected CEM cells rescues cells from the otherwise lethal infection and permits cell proliferation in long-term cultures.

This *in vitro* evidence suggests that selected fluoroquinolones might be considered in clinical trials as possible AIDS drugs. However, it is always difficult to predict potential *in vivo* clinical effects solely on the basis of *in vitro* data. While these drugs have been proven clinically safe (9), they were evaluated primarily for antibiotic activity. The current data suggest that AIDS patients being treated with fluoroquinolones as antibacterial agents should be followed not only for inhibition of opportunistic infections, but also carefully observed from the viewpoint of improvement of immune

deficiency.

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