Quinoline compounds decrease *in vitro* spontaneous proliferation of peripheral blood mononuclear cells (PBMC) from human T-cell lymphotropic virus (HTLV) type-1-infected patients

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Abstract

*In vitro* spontaneous proliferation is the immunological hallmark of peripheral blood mononuclear cells (PBMC) from HTLV-1-infected individuals. Quinoline compounds down regulate *in vitro* cell proliferation of HTLV-1 transformed cell lines. In the present study we assessed the capacity of quinolines to inhibit spontaneous cell proliferation of PBMC from HTLV-1-infected individuals. Twenty-two quinolines were evaluated. Toxicity was first assessed on PBMC from healthy donors by using both the Trypan blue technique and Tetrazolium Salt (XTT) method and then the antiproliferative effect was measured by a classic lymphoproliferative assay on PBMC from three HTLV-1-infected individuals, in the presence of decreasing concentrations of quinolines (from 100 μM to 0.8 μM), after 5 days of culture.

We found that 14 out of 22 compounds were non-toxic to PBMC from uninfected individuals at 100, 50 and 10 μM. Four compounds presented a capacity to inhibit more than 80% of the spontaneous proliferation: 7 at 25 μM and 10, 20 and 23 at 100 μM. Our results indicate that some quinolines block spontaneous proliferation of PBMC from HTLV-1-infected individuals.

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1. Introduction

Human T-lymphotropic virus (HTLV) type 1 is a human retrovirus that causes a wide spectrum of diseases affecting about 10–20 million people worldwide. There are large endemic foci of HTLV-1 infection in Japan, Caribbean, Melanesia, Central and West Africa and South America [1]. Brazil has the highest absolute number of HTLV-1-infected individuals and the prevalence is highest in Salvador, a city in the Northeast of Brazil, where 2% of inhabitants are infected [2].

HTLV-1 is the etiologic agent of HTLV-1-associated myelopathy/tropical spastic paraparesis (HAM/TSP) [3,4] and adult T-cell leukemia/lymphoma (ATLL) [5]. HTLV-1 is also associated with many other diseases such as child-infective dermatitis [6], tuberculosis [7], strongyloidiasis [8] but it appears that 90% of those infected remain asymptomatic. There are no known effective drugs that treat the HTLV-1 infection. Vitamin C has been shown to modulate the symptoms of HAM/TSP [9]. Antiretroviral drugs such as nucleoside reverse transcriptase inhibitors have inhibited replication of HTLV-1 *in vitro* [10,11] and *in vivo* [12,13]. Nevertheless, the high cost of this treatment limits its application, particularly in developing countries.
Recently, quinoline compounds have demonstrated an inhibitory effect on proliferation of HTLV-1 transformed cell lines [14,15]. These quinolines are either alkaloids isolated from Galipea longiflora, a Bolivian plant with medicinal properties [16] or were synthetically produced [17,18]. Several quinoline derivatives have shown very promising activity against several parasitic infections including cutaneous and visceral leishmaniasis [19], Plasmodium vinckei petteri [20] and experimental Trypanosoma cruzi infection in mice [21]. In addition, these compounds inhibited the HIV-1 integrase activity [22].

In this study, we have evaluated the inhibitory effect of 22 new or previously evaluated quinolines on spontaneous proliferation of PBMC from HTLV-1-infected individuals. We hope that some of these compounds may eventually be developed into therapies used clinically for HTLV-1.

2. Materials and methods

2.1. Patients

Samples were obtained from 24 asymptomatic HTLV-1-infected individuals from The HTLV-1 Reference Development of Science Foundation and 11 healthy individuals from Bahia State Blood Bank (HEMOBA). Both centers are located in Salvador, Bahia — Brazil. All samples were screened for HTLV-1/2 antibodies by enzyme-immune assay (ELISA) (Ab-Capture ELISA Test System — Ortho-Clinical Diagnostics, Inc., Raritan, New Jersey) and confirmed by Western blot assay (HTLV Blot 2.4, Genelabs Technologies, Singapore). The Ethical Board of Oswaldo Cruz Foundation (FIOCRÚZ) approved this study, and informed consent was obtained from all enrolled patients.

2.2. Quinolines

Compounds 2–23 were prepared according to previously published methods (see Fig. 1). 2-propenyl-quinoline 5 was obtained from quinoline 1a as already reported [23]. Compounds 2, 3, 6, 12, were obtained by treatment of 2-quinidine 1c with the appropriate aldehyde in acetic anhydride [17]. Compound 13 was obtained by treatment of 8-hydroxy-2-methyl quinoline 1b by butyllithium followed by addition of ethanol, as reported in Ref. [24]. Compounds 17 and 21 were obtained as well, when 2-methyl quinoline (2-quinidine) 1c was treated by butyllithium followed by addition of ethanol, or benzaldehyde, respectively. Treatment of 2-quinidine 1c by butyllithium followed by addition of dimethoxyglyoxaldehyde, afforded compound 8, which under oxidation conditions afforded compound 9. Ethylmagnesium bromide addition to 2-quinaldehyde 1d afforded the corresponding alcohol 11 (as reported in Ref. [17]), whereas Wittig and Wittig–Horner reactions with 1d gave rise to the expected vinylic derivatives 10 and 22 (as reported in Ref. [18]). The alkynyl derivatives 04, 14–16 were obtained through the palladium catalyzed coupling reaction between the corresponding alkynes and the required 2- and 3-halogenated quinolines 1e and 1f. Then compound 18 was obtained by TBAF treatment of 4. N-oxides 19 and 20 were obtained by H₂O₂ oxidation of the corresponding compounds 1g and 1h. Aldehyde 23 was obtained by reduction of ester 22, whereas alcohol 7 was obtained by Dibal reduction of 23. All spectroscopic data of the synthesized products were in agreement with the proposed structures. Chemical structures of quinolines evaluated in this study are shown in Fig. 1. They were dissolved in dimethyl sulfoxide (DMSO) and sterilized by gamma irradiation at a dose of 65 000 rads and stored at –20 °C until use.

2.3. Cell cultures

PBMC were obtained by Ficoll-Hypaque density gradient centrifugation (Pharmacia, Biotech Uppsala, Sweden) from heparinized venous blood. To evaluate the cell toxicity of quinolines, PBMC from healthy controls were first stimulated for 3 days with 2 µg/ml purified phytohaemagglutinin (PHA) and subsequently cultured with 2 U/ml human recombinant IL-2 (2 U/ml) (Sigma Chemical Co., St. Louis, MO, USA) in RPMI 1640 medium supplemented with 2 mM l-glutamine, 1% non-essential amino acids, 1 mM sodium pyruvate, 100 U/ml penicillin, 100 µg/ml streptomycin and 5% human AB serum (all provided by Sigma Chemical Co., St. Louis, MO, USA), for 5 days.

2.4. Cellular toxicity assays

2.4.1. Trypan blue

Toxicity tests were performed using PBMC from three different healthy controls and PBMC from three HTLV-1-infected individuals. In 96-well U-bottom culture plates 1 × 10⁵ cells/well were cultured in triplicate (Costar, Cambridge, MA), in the presence of three concentrations of quinolines (10 µM, 50 µM, and 100 µM) for 3 days, at 37 °C in a 5% CO₂ humidified atmosphere. Cells were counted using a light microscope (Nikon-Model Labophot-2, Japan). To determine the percentage of viable cells, the number of unstained cells were divided by the total number of cells and then multiplied by 100. Cellular viability of at least 85% was considered to indicate a nontoxic compound. This value was arbitrarily chosen based on our Trypan blue experiments with non-infected cells in the presence of medium without compound which yielded values of 97 ± 4% viable cells.

2.4.2. XTT assay

PBMC were plated in 96-well plates at 1 × 10⁵ cells/well. Serial dilutions of compounds were added to the wells. Final concentration of DMSO in all wells was at 1%, in a total volume of 200 µl. The plates were maintained in a 37 °C incubator. After 3 days, 50 µl of XTT—PMS solution (20 µM) was added to each well. Then, the plate was incubated at 37 °C for 4 h. The optical density (OD) was determined by V-max photometer ( Molecular Devices, Inc., Menlo Park, CA) at a test wavelength of 450 nm. The toxicity represents the ratio of OD of a well in the presence of quinolines with the OD of control wells in the presence of medium containing DMSO. The cellular viability of at least 85% was considered to indicate a non-toxic compound.
2.4.3. Cellular proliferation assay

To evaluate the effects of quinoline compounds on cellular proliferation, PBMC from HTLV-infected patients were cultured in supplemented RPMI 1640 culture medium with 5% AB serum, in 96-well U-bottom culture plates (Costar, Cambridge, MA), in triplicate, at 37 °C in a 5% CO₂ humidified atmosphere. Briefly, $1 \times 10^5$ cells/well were cultured in the presence of serial dilutions (100 to 0.8 μM) of quinolines. After 5 days of culture, cells were pulsed overnight with 1 μCi 3H-thymidine (specific activity 2 Ci/mMole; ICN, Costa Mesa,
CA). Incorporated 3H-thymidine was measured with a liquid scintillation beta counter (Direct Beta Counter, Matrix 9600 — Packard). Results were expressed as mean counts/min. The stimulation index (SI) represents the ratio of mean counts obtained in the presence of quinolines and mean counts obtained without quinolines. Percent inhibition was determined by comparing cell counts in wells containing compounds to those in wells containing cells cultured with medium.

2.5. Statistical analyses

Correlation between continuous variables was examined by Spearman test. Differences were considered significant when p < 0.05.

3. Results

From 22 compounds assessed, 14 were non-toxic in all tested concentrations (3, 5, 8–11, 14, 15, 17–21, 23). Three were non-toxic at 50 and 10 µM (6, 7, 16), and three were non-toxic only at 10 µM (2, 4, 22). High toxicity in all tested concentrations was observed in 13 and 14. Viability assessed using the Trypan blue and XTT method was similar for all quinolines at all tested concentrations (Fig. 2). Then, we tested the inhibitory capacity of compounds on spontaneous proliferation of PBMC from HTLV-1-infected individuals. Nineteen compounds at non-toxic concentrations were evaluated: 2–11, 14, 15, 17–23 (Table 1). Four compounds had a capacity to inhibit more than 80% of the spontaneous proliferation: 10, 20 and 23 at 100 µM, and 7 at 25 µM. The cellular toxicity assessed by Trypan blue in presence of quinoline compounds was 82% to 7 at 25 µM, 81% to 10 and 23, and 77% to 20 at 100 µM, as shown in Fig. 3. In the absence of quinoline compounds, the mean of cellular viability of PBMC from HTLV-1-infected individuals was 85 ± 5%. These experiments suggested that the inhibitory effect of quinoline compounds on spontaneous proliferation of PBMC from HTLV-1-infected individuals was not due to toxicity.

4. Discussion

In our study, we identified four quinoline compounds with high inhibitory activity on the spontaneous proliferation of PBMC from HTLV-1-infected individuals. In a previous study, three of these quinoline compounds were able to inhibit HTLV-1 transformed cell line (HUT-102) [14]: 7, 20 and 23 (which correspond to 13, 19, 4, respectively [14]). Compound 7, possessing one hydroxyl at the 8-position on the heteroaromatic ring, and another hydroxyl at the 2-position of the propyl chain and 20, a 2-styrylquinoline containing an N-oxo-quinoline ring (which correspond to 13 and 19, respectively [14]) inhibited >80% of both HUT-102 cell line and spontaneous proliferation of PBMC from HTLV-1-infected individuals. Compound 23 possessing a conjugated formyl group to the ethenyl chain (which corresponds to compound 4 [14]) had a high antiproliferative effect on PBMC from HTLV-1-infected individuals, whereas its effect on HUT-102 HTLV-1 cell line was moderate [14]. Two other compounds, 12 and 18 (which correspond to 7 and 21, respectively [14]) were also assessed. Compound 12, which possesses both hydroxyl and conjugated instauration, had no antiproliferative activity on HUT-102 cell line and had toxicity to PBMC from HTLV-1 infected individuals. Compound 18 had low to moderate antiproliferative effect to both HUT-102 cell line and to PBMC from HTLV-1 infected individuals [14].

Quinoline compounds could use different pathways to inhibit PBMC spontaneous proliferation from HTLV-1-infected individuals or of cell lines. Proliferation of cell lines is due to a monoclonal expansion whereas spontaneous proliferation of PBMC from HTLV-1-infected individuals is polyclonal [25,26]. Spontaneous proliferation induced by HTLV infection is observed in up to 50% of cultures of PBMC from HTLV-1 carriers. Although the role of spontaneous proliferation on pathogenesis of this infection is not well established, individuals who have developed HAM/TSP show an even higher degree of spontaneous proliferation concomitant with increased transcription of the HTLV-1 provirus [27]. This phenomenon is due, in part, to an IL-2/IL-2R autocrine loop. It was shown

![Fig. 2. Correlation between cellular viability obtained by Trypan Blue (% viable cells) and XTT methods (optic density). Twenty-two quinoline compounds were evaluated. Each point represents one compound tested at a concentration of 100 µM. Correlation of Spearman (p < 0.005).](image-url)

Table 1

<table>
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<th># Quinoline</th>
<th>Inhibition (%)</th>
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<th>Inhibition (%)</th>
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<tr>
<td>2</td>
<td>50 µM: 3</td>
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<tr>
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<tr>
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<td>11</td>
<td>100 µM: 10</td>
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<td>100 µM: 84</td>
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ND: not done.
that the addition of monoclonal antibodies against IL-2 or IL-2Rα partially inhibited the spontaneous proliferation. The mechanisms involved in the inhibition of spontaneous proliferation by the quinoline compounds are not clear. Therefore, studies are necessary to elucidate the mechanisms of action of these drugs. Particularly, we are conducting studies to evaluate the effect of quinoline compounds on cytokine production, such as IL-10, TNF-α and IFN-γ, on the HTLV proviral load and on induction of apoptosis.

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References


