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Research paper

Antimalarial drugs trigger lysosome-mediated cell death in chronic lymphocytic leukemia (CLL) cells

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ABSTRACT

Lysosomes are the most acidic vesicles within mammalian cells and are promising targets for the treatment of breast cancer, glioblastomas and acute myeloid leukemia (AML). Our previous studies have shown that chronic lymphocytic leukemia (CLL) cells are also sensitive to lysosome disruption and cell death, by siramesine or chemotherapy. In the present study, we screened the antimalarial drugs, mefloquine, atovaquone, primaquine, and tafenoquine, for their effects on lysosome disruption and cytotoxicity in primary CLL cells. We found that mefloquine and tafenoquine could permeabilize lysosome membranes and induce cell death at doses that are clinically achievable. In contrast, these agents had less effect on normal B cells. Tafenoquine was most effective at inducing cell death, and this was associated with increased formation of reactive oxygen species (ROS) and lipid peroxidation. Addition of ROS scavengers blocked both tafenoquine- and mefloquine-induced cell death. Moreover, blocking the activity of cathepsins released from the lysosomes decreased tafenoquine-induced cell death. Taken together, lysosome disruption using antimalarial drugs is a novel approach for the treatment of CLL.

1. Introduction

Chronic lymphocytic leukemia (CLL) is the most common leukemia and is characterized by the accumulation of monoclonal B cells with typical immunophenotype in peripheral blood, marrow, lymph nodes, and spleen [1]. Patient outcomes are heterogeneous with unmutated status of the immunoglobulin heavy chain variable region (*IGHV*) and TP53 disruption being important poor prognostic markers. While chemoimmunotherapy remains standard first-line treatment for most patients, the B-cell receptor pathway inhibitors, ibrutinib and idelalisib, or the bcl-2 antagonist, venetoclax, are highly effective in chemotherapy-refractory patients and for those with TP53 dysfunction [2,3]. Nevertheless, despite these therapies, CLL remains incurable except for fit younger patients who undergo and survive a marrow stem cell transplant.

Identification of therapeutic strategies that selectively target CLL cells and can improve the therapeutic index of existing therapies are required. We have previously demonstrated that CLL cells have high lysosome content compared to normal B cells and treating CLL cells with siramesine, a lysosome disrupting drug, selectively induces apoptosis in these cells [4]. Moreover, even CLL cells with a deletion 17p are sensitive to siramesine. In addition, the increased efficacy of obinutuzumab over rituximab has been attributed to its unique ability to enter and lyse lysosomes in CLL cells [5]. In other malignancies, such as lung or breast cancer, lysosome targeting drugs are also effective at inducing cell death [6–8]. Thus, disrupting lysosomes is a potential therapeutic approach for the treatment of CLL.

Lysosomes can activate programmed cell death pathways through the release of lysosomal hydrolases, such as cathepsins [9,10], by a process termed lysosomal membrane permeabilization (LMP) [11–15]. In acute myeloid leukemia (AML) cells, the antimalarial drug mefloquine causes the release of cathepsins from lysosomes leading to protein degradation, mitochondrial dysfunction, increased reactive oxygen species (ROS) levels and apoptosis [8]. LMP can also be induced by the inhibition of acid sphingomyelinase (ASM), using agents such as siramesine [16,17]. This leads to lysosome disruption, increased ROS levels and cell death. Siramesine is effective at inducing cell death in CLL cells, but is not FDA approved, nor is it under clinical development

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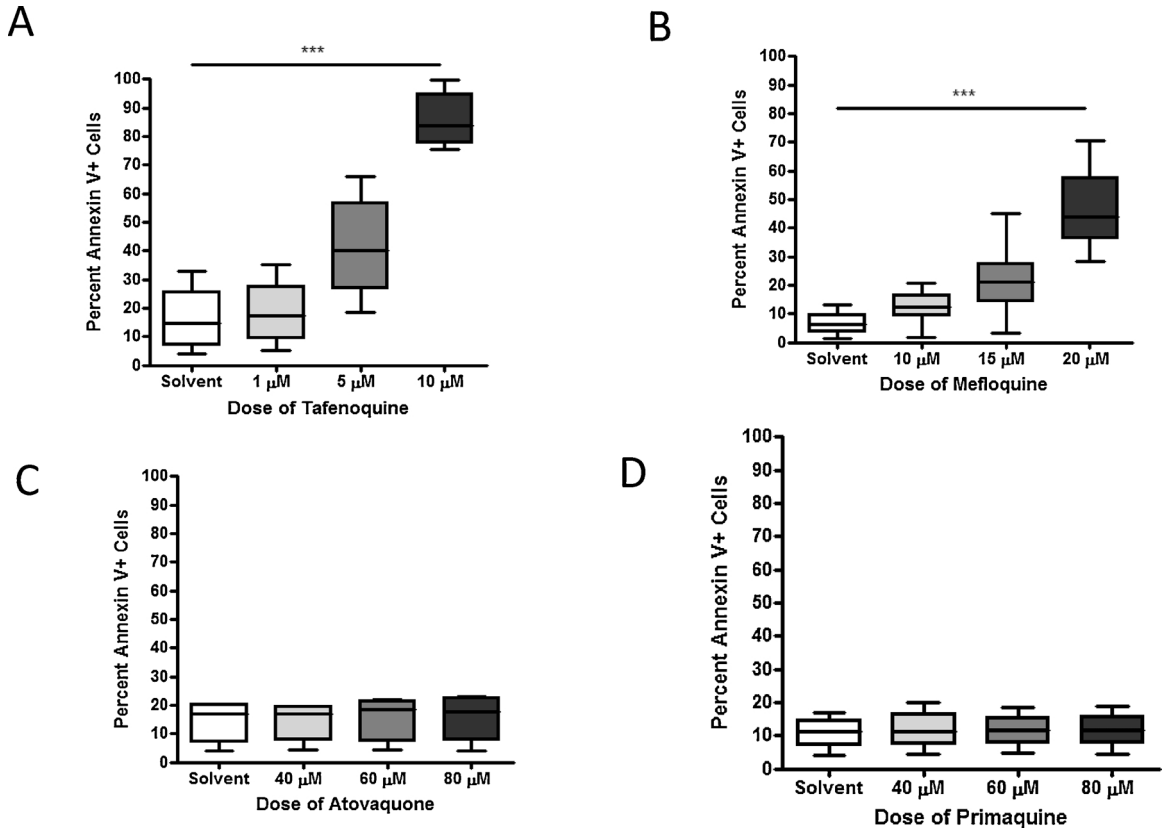

Fig. 1. CLL cells are sensitive to tafenoquine and mefloquine.
CLL cells were treated with increasing doses of tafenoquine (A), mefloquine (B), atovaquone (C) or primaquine (D) for 24 h. The cells were then stained with Annexin V-FITC and 7AAD and examined by flow cytometry. Cell death was defined by both Annexin V and 7AAD positive cells. The bar represents the mean and standard errors for 5 CLL patient samples. *** represents statistical significance of $P < 0.05$.

to treat cancer [4]. Therefore, in the present study, we investigated FDA approved antimalarial drugs for their ability to induce LMP in CLL and evaluated their mechanism of action.

2. Materials and methods

2.1. Cell culture

Peripheral blood samples were collected from patients following informed consent in accordance with the Research Ethics Board at the University of Manitoba. Samples were mixed with RosetteSep (Stem Cell Technologies, Vancouver, BC, Canada) if the lymphocyte count was less than 40×10^9/l and then purified on a Ficoll-Paque gradient (GE Healthcare, Mississauga, ON, Canada). Red blood cells were lysed with RBC lysis buffer (eBioscience, San Diego, CA, USA). All blood samples were processed within 24 h after collection and used fresh. Patient samples were only excluded from the study on the basis of low cell yield after processing or low viability. Samples were randomly selected for different experiments. Laboratory personnel were blinded to patient characteristics until after all experiments had been performed. The human bone marrow-derived stromal cell line HS-5 (obtained as a kind gift from Dr Peng Huang, MD Anderson Cancer Centre) were cultured in DMEM with high glucose (Hyclone, GE Healthcare) and 10% fetal bovine serum (Hyclone, GE Healthcare), with penicillin and streptomycin (Gibco, Life Technologies). Stromal cells were seeded at 5×10^4 cells per well of a 48-well plate 24 h prior to the addition of 5×10^6 CLL cells. This represents a 1:100 HS-5: CLL co-culture ratio. Co-cultures were maintained in a humid 37 °C incubator for 24 h prior to the addition of drug.

2.2. Drugs and stimuli

Tafenoquine, mefloquine, and atovaquone (Sigma, Oakville, ON, Canada) were dissolved in dimethyl sulfoxide (DMSO) and kept at room temperature. D-Sphingosine (Sigma) was dissolved in DMSO and stored in single-use aliquots at −20 °C. Various inhibitors were added 1 h prior to drug treatment: α-tocopherol (Sigma) was dissolved in ethanol and prepared fresh for each experiment; lycopene (Sigma) was dissolved in ethanol; N-acetyl cysteine (Sigma) was dissolved in 1 × phosphate-buffered saline at pH 7.4 and made fresh for each experiment; glutathione (Sigma) was dissolved in 1 × phosphate buffered saline and made fresh for each experiment; Ca-074-Me (Enzo Life Sciences, Farmingdale, NY, USA) was dissolved in DMSO; Chymostatin (Sigma) was dissolved in DMSO; E64 (Sigma) was dissolved in water; zVADfmk (Caspase Inhibitor VI, Millipore, Etobicoke, ON, Canada) was dissolved in DMSO, and SKI II (Sigma) was dissolved in DMSO. Unless preparations were made fresh, drug stocks were frozen at −20 °C.

2.3. Flow cytometry

Cells were stained with Annexin V-FITC (BD Biosciences, San Jose, CA, USA) and 7AAD (BD Biosciences) for 15 min at room temperature for apoptosis analysis. For lysosome staining, cells were incubated with 50 nM Lysotracker Red DND-99 (Invitrogen, Waltham, MA, USA) for 30 min at 37 °C. For mitochondrial membrane potential and soluble reactive oxygen species analysis, cells were stained with 25 nM DIOC6 (Sigma) and 3.2 μM DHE (Sigma) together for 30 min at 37 °C. For lipid peroxidation analysis, cells were stained with 1 μM BODIPY 581/591 (Invitrogen) for 30 min at 37 °C. Prior to analysis, all stained cells were diluted in 1 × phosphate-buffered saline or 1 × Annexin V Binding Buffer (Invitrogen).