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Lysosomotropic cationic drugs induce cytostatic and cytotoxic effects: Role of liposolubility and autophagic flux and antagonism by cholesterol ablation

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ABSTRACT

Cation trapping in acidic cell compartments determines an antiproliferative effect that has a potential interest in oncology, as shown by clinical data and trials involving chloroquine and hydroxychloroquine. To further characterize the mechanism of this effect, we studied a series of 6 substituted triethylamine (s-Et_3N) drugs that encompasses a wide range of liposolubility (amiodarone, quinacrine, chloroquine, hydroxychloroquine, lidocaine, and procainamide). Three tumor cell lines and primary human endothelial cells were exploited in proliferation assays (48 h, cell counts). Accumulation of the autophagic effector LC3 II and the apoptotic marker cleaved PARP1 (immunoblots), cytotoxicity, cell cycle analysis and endocytic function were further tested in the p53-null histiocytic lymphoma U937 line. A profound and desynchronized antiproliferative effect was observed in response to all s-Et_3Ns with essentially no cell type specificity. Predictors of s-Et_3N potency were liposolubility and the acute accumulation of the autophagic effector LC3 II (6 h-treatments). For each s-Et_3N, there was an antiproliferative concentration range where cytotoxicity and apoptosis were not triggered in U937 cells (24–48 h-treatments). Quinacrine was the most potent cytostatic drug (1–5 μM). Co-treatment of cells with inhibitors of cholesterol, β-cyclodextrin or lovastatin, partially reversed the antiproliferative effect of each s-Et_3N. The cytopathology induced by cationic drug accumulation includes a cytostatic effect. Its intensity is cell type- and p53-independent, but predicted by the inhibition of autophagic flux and by the liposolubility of individual drugs and alleviated by cholesterol ablation. The superiority of quinacrine, biomarker value of LC3 II and antagonism by a statin may be clinically relevant.

1. Introduction

Lysosomotropic drugs can be defined as a series of weak bases that concentrate in acidic organelles, primarily the lysosomes and late endosomes, following their protonation at low pH and slow retrodiffusion under their cationic form; the proton pump V-ATPase provides the energy for this pseudo-transport mechanism (Marceau et al., 2012). The interruption of autophagosome clearance ensues in cells, and an antiproliferative effect is observed. A novel field of application of autophagic flux inhibitors is clinical oncology: hydroxychloroquine currently undergoes clinical trials for various solid and hematologic cancers (Sehgal et al., 2015). Chloroquine combination with conventional chemotherapy increased survival in patients with glioblastoma multiforme (Briceno et al., 2007). However, autophagy has context-dependent roles in cancer development and may be protective, especially early in the course of the disease (Thoburn et al., 2014).

Despite these exciting developments, little is known about the mechanisms and determinants of the anti-proliferative action of autophagic flux inhibitors. A constant feature of the cytopathology induced by cation accumulation in acidic vacuoles is an inhibition of cell proliferation without important cytotoxicity; we have observed this in various cellular models in response to lidocaine (Bawolak et al., 2010); 2-dimethylaminoethanol (Morissette et al., 2007), triethylamine (Et_3N) and procainamide (Morissette et al., 2004, 2005), as well as tamoxifen in an estrogen receptor-negative cell line (Marceau et al., 2012). Golden et al. (2015) recently analysed the cytotoxic effect of a series of anti-malarial drugs: all were autophagic flux inhibitors of varying potency and they induced apoptosis in human glioma cell lines independently of p53. Phospholipidosis is the late cytopathologic reorganization of vacuoles that have sequestered cationic drugs; in the affected cells, several genes that control lipid synthesis are upregulated (Sawada et al., 2005; Nioi et al., 2007). A new hypothesis about the antiproliferative effect of a lysosomotropic drug emerged in studies of leelamine, a novel lipophilic cationic agent: vacuolar cholesterol accumulation and interruption of vesicular cycling were observed (Kuzu et al., 2014). Cholesterol extraction using β-cyclodextrin reversed

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the antiproliferative effect and the depression of vacuolar traffic in that study.

We hypothesized that the antiproliferative effect of lysosomotropic drugs (1) is a universal response to amines susceptible to ion trapping; (2) possesses a uniform mechanism, mainly cytostatic and related to vacuolar alterations, and (3) exhibits a potency inversely correlated to their lipophilicity, as this physicochemical property clearly predicts the concentrations for which the cytopathology is observed (Marceau et al., 2012). To address these issues, we exploited a previously defined series of substituted triethylamine (s-Et_3N) drugs that span the whole lipophilicity scale (Suppl. Fig. 1) and that all were shown to be concentrated in a V-ATPase-dependent manner (Marceau et al., 2012). They include presently or formerly clinically used therapeutic agents but in classes that bear no obvious relationship with oncology (3 antimalarial, 2 anti-arrhythmic and one local anesthetic drugs). Cell models used in the analysis were selected to isolate any cell-type specific effects; further, the mechanism of antiproliferative effects was characterized in a p53-null histiocytic lymphoma cell line.

2. Methods

2.1. Cell culture

The human melanoma M21 cell line, originally obtained from Dr. David Cheresh (The Scripps Research Institute, San Diego, CA), was a gift from Dr. Eric Petitclerc (Héma-Québec, Québec, Canada); it is tumorigenic in immunodeficient mice (McMahon et al., 2001). Most melanoma cells are extremely radioresistant and typically express non-mutated p53 protein; DNA-damaging agents lead to accumulation of p53 but not to apoptosis in these cells, as modeled by the M21 line (Bao and Strömblad, 2004). M21 and HEK 293a cells, originally obtained from Sigma-Aldrich, were cultured in DMEM supplemented with 5 and 10% fetal bovine serum, respectively, and antibiotics. The institutional research ethics board approved the anonymous use of human umbilical cord segments obtained after caesarean sections. Human umbilical vein endothelial cells (HUVECs) were isolated by collagenase digestion of umbilical veins from undamaged sections of fresh cords and cultured as described (Koumbadinga et al., 2010). The cells were maintained and passaged in Endothelial Cell Growth Medium (EGM, Lonza-Clonetics, Basel, Switzerland) used with the supplied growth supplement (final fetal bovine serum concentration 2%) and antibiotics. HUVECs express functional p53 (Zhang et al., 2011). Human monocytic leukemia cells (U937) were originally isolated from the histiocytic lymphoma of a 37-year-old male patient and were grown in RPMI 1640 medium (GIBCO) supplemented with 10% fetal bovine serum. They are p53 null due to a large deletion in both copies of the *p53* gene (Shiohara et al., 1994; Oliveiro et al., 1997).

2.2. Cell proliferation and counting

The study of the antiproliferative effects of s-Et_3N drugs was made utilizing a previously applied proliferation assay in cellular models.

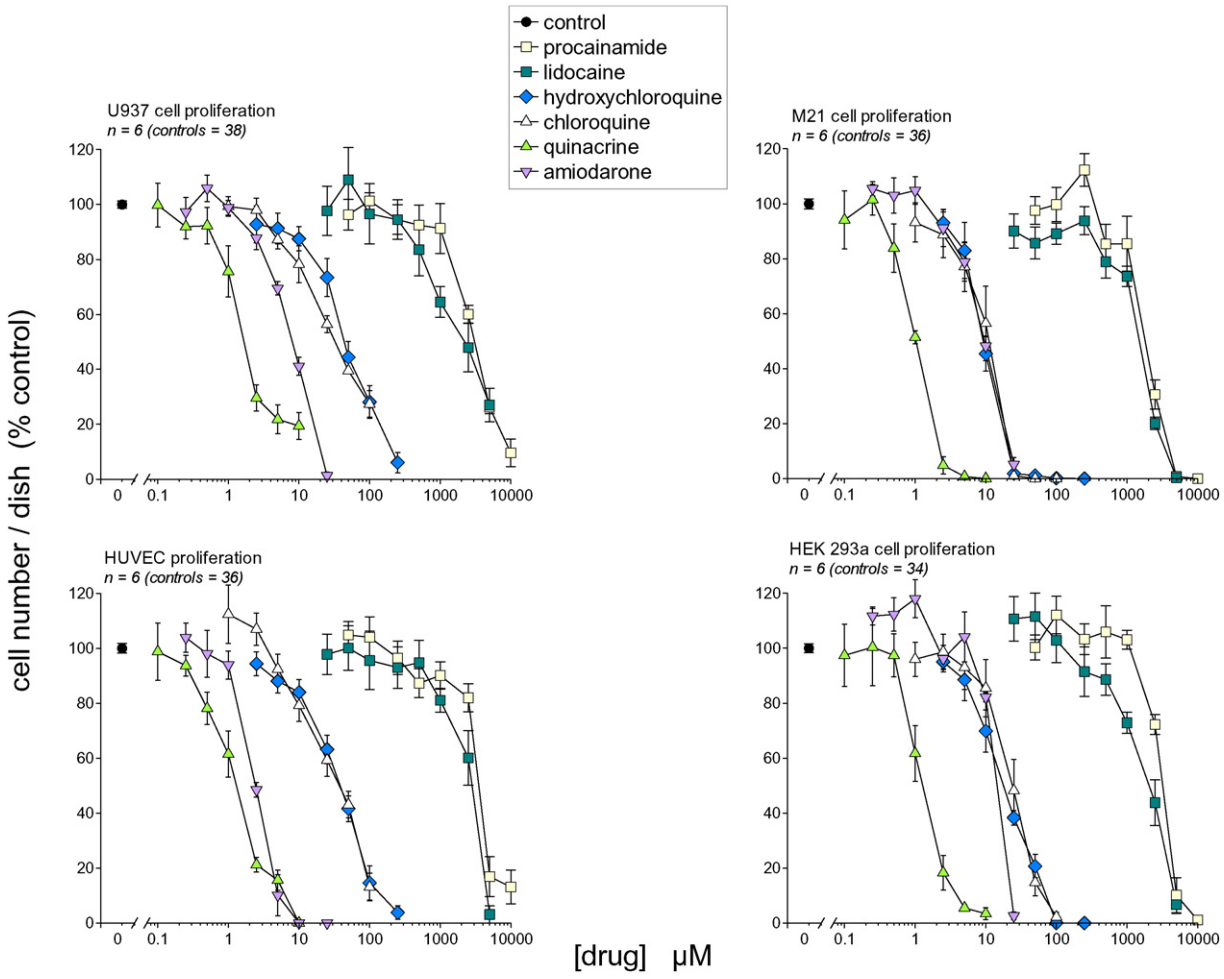

Fig. 1. Effect of substituted trimethylamine (s-Et_3N) drugs on the proliferation of 4 cultured cell types. 50,000 cells were seeded in petri dishes 72 h before counts in the serum-containing medium appropriate for each cell type (drugs present for the last 48 h). Cell counts are normalized as a percent of the control values recorded in each day of experiments. The absolute control counts at 72 h were 946,592 ± 51,976 (n = 38) for U937 cells, 484,597 ± 24,501 (n = 36) for M21 cells, 308,315 ± 9089 (n = 36) for HUVECs and 528,015 ± 22,496 (n = 34) for HEK 293a cells.