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Invited Review

Effect of lysosomotropic molecules on cellular homeostasis

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ABSTRACT

Weak bases that readily penetrate through the lipid bilayer and accumulate inside the acidic organelles are known as lysosomotropic molecules. Many lysosomotropic compounds exhibit therapeutic activity and are commonly used as antidepressant, antipsychotic, antihistamine, or antimalarial agents. Interestingly, studies also have shown increased sensitivity of cancer cells to certain lysosomotropic agents and suggested their mechanism of action as a promising approach for selective destruction of cancer cells. However, their chemotherapeutic utility may be limited due to various side effects. Hence, understanding the homeostatic alterations mediated by lysosomotropic compounds has significant importance for revealing their true therapeutic potential as well as toxicity.

In this review, after briefly introducing the concept of lysosomotropism and classifying the lysosomotropic compounds into two major groups according to their cytotoxicity on cancer cells, we focused on the subcellular alterations mediated by class-II lysosomotropic compounds. Briefly, their effect on intracellular cholesterol homeostasis, autophagy and lysosomal sphingolipid metabolism was discussed. Accordingly, class-II lysosomotropic molecules inhibit intracellular cholesterol transport, leading to the accumulation of cholesterol inside the late endosomal-lysosomal cell compartments. However, the accumulated lysosomal cholesterol is invisible to the cellular homeostatic circuits, hence class-II lysosomotropic molecules also upregulate cholesterol synthesis pathway as a downstream event. Considering the fact that Niemann-Pick disease, a lysosomal cholesterol storage disorder, also triggers similar pathologic abnormalities, this review combines the knowledge obtained from the Niemann-Pick studies and lysosomotropic compounds. Taken together, this review is aimed at allowing readers a better understanding of subcellular alterations mediated by lysosomotropic drugs, as well as their potential therapeutic and/or toxic activities.

Contents

Abbreviations: AO, acridine orange; ATP, adenosine triphosphate; ASMD, acid sphingomyelinase deficiency; CAD, cationic amphiphilic drugs; CMap, connectivity map database (www.broadinstitute.org/cmap/); DMSO, dimethyl sulfoxide; ER, endoplasmic reticulum; HDL, high-density-lipoprotein; LDL, low-density-lipoprotein; MDR, multidrug resistant; NPC, Niemann-Pick Type-C disease; SREBP, sterol regulatory element-binding protein; SSRI, selective seratonine receptor inhibitor; LE/L, late endosomal/lysosomal.

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1. Introduction

Weakly basic amine compounds that induce rapid vacuolization of cells are known as lysosomotropic molecules [1–3]. "Lysosomotropism" was first introduced by de Duve et al. to define compounds that accumulate up to several hundred-fold higher concentrations within the late endosomal/lysosomal (LE/L) cell compartments [1]. Weakly basic compounds, depending on their lipophilicity, can readily diffuse through the limiting membrane of acidic organelles in their unionized form and get protonated (ionized) in the acidic lumen (Fig. 1). Due to their decreased membrane permeability, protonated molecules cannot cross-back to the cytosol, hence get trapped and accumulate inside the acidic lumen (also referred as ion trapping) [4]. Although they have diverse structures, most of the lysosomotropic compounds harbor an amine group that is responsible for their weakly basic properties. The degree of ion trapping depends on pH of the cellular compartment as well as physicochemical properties of the compound such as pKa (acid dissociation constant) and membrane permeability. However, in addition to ion trapping, other mechanisms may also be playing a role in the lysosomotropism, as it was reported that experimentally observed accumulation was several fold higher in contrast to theoretically predicted amount [5,6]. As a matter of fact, some weakly basic molecules fail to show lysosomotropism even though their biochemical properties are favorable [7]. Therefore, further studies are required to identify other mechanisms that contribute to lysosomotropism.

Lysosomes, endosomes and golgi apparatus are the major acidic organelles of the mammalian cells. As a digestion and recycling center, lysosomes contain over fifty different hydrolytic proteases such as glycosidases, sulfatases, nucleases and lipases, which have optimal activity in acidic lumen of the organelle [8]. On the other hand, endosomes are compartments of the endocytic transport system that is primarily involved in internalization of material from the plasma membrane [9]. They function as a sorting compartment

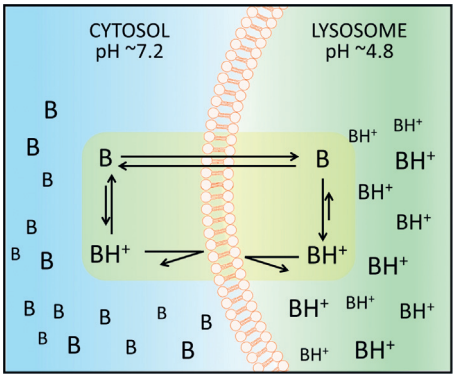

Fig. 1. Protonation of weakly basic amines triggers their accumulation in acidic organelles. Lipophilic, weak bases can readily diffuse through membranes in their neutral form (B). However, upon protonation (BH^+) in acidic environments, they lose their membrane permeability and become trapped in the lumen of these organelles.

where ingested material is sorted prior to reaching lysosomes for degradation. Both lysosomes and endosomes have a unique membrane structure that consists of intraluminal membranes and a layer of external limiting membrane. The acidic lumen of both organelles is established by the activity of the vacuolar H^+ ATPase (v-ATPase) protein that transports protons (H^+) across the external membrane [8]. The difference between the pHs of the lumen and cytosol positively correlates with accumulation of protonated amine in the acidic lumen. Therefore, v-ATPase inhibitors such as Bafilomycin A1 suppress lysosomotropism by increasing the pH of acidic organelles [10].

Lysosomotropic compounds trigger cellular vacuolization following their treatment [4,11]. Even though the details of this vacuolar response is not well understood, late endosomal, lysosomal and trans-Golgi specific proteins co-localize with these vacuoles [4,12]. It has been hypothesized that these vacuoles could be greatly expanded hybrid organelles that were formed by fusion of lysosomes with late endosomes through trafficking in a retrograde manner [3]. Although there is still a need for further experiments to confirm the identity and origin of these vacuoles, induction of vacuolization is the most notable feature of lysosomotropic agents.

2. Classification of lysosomotropic compounds

Lysosomotropic compounds can be classified into two major groups based on their cytotoxic activity. Hydrophilic lysosomotropic molecules such as ammonium (NH_3), methylamine (CH_3NH_2), ethylamine ($CH_3CH_2NH_2$) can be classified as class-I compounds, while hydrophobic or amphiphilic lysosomotropic molecules, with at least one or more hydrophobic rings and a hydrophilic tail (generally harbors a polarizable amine group) can be classified as class-II compounds (Table 1 and Fig. 2). Many tricyclic antidepressants (e.g.,imipramine, nortriptyline, amitriptyline), phenothiazine antipsychotics (e.g., promazine, fluphenazine, perphenazine), antihistamines (e.g., desloratadine, promethazine), SSRIs (Selective Serotonin Reuptake Inhibitors) (e.g., sertraline, fluvoxamine, fluoxetine), U18666A, leelamine, monodansylcadaverine, acridine orange and chloroquine can be given as the examples of class-II lysosomotropic compounds.

Class-I lysosomotropic compounds are generally well-tolerated by cells. Although they induce massive vacuolization, they do not trigger cell death up to millimolar concentrations (Fig. 2). At these high concentrations, hydrophilic lysosomotropic compounds accumulate inside the lumen of the acidic organelles and raise the luminal pH leading to disruption of the lysosomal pathway of protein degradation [13–15].

On the other hand, class-II lysosomotropic compounds are significantly more toxic to the cells and able to induce cell death in micromolar concentrations. Hydrophobic portion of these amphiphilic compounds allows their accumulation in the internal membranes of the lysosomes, causing perturbation of the activity of lysosomal membrane proteins such as acid sphingomyelinase,