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One man's poison is another man's meat: Using azithromycin-induced phospholipidosis to promote ocular surface health

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ABSTRACT

Drug-induced phospholipidosis (PLD) is a common adverse effect which has led to the termination of clinical trials for many candidate pharmaceuticals. However, this lipid-inducing effect may be beneficial in the treatment of meibomian gland dysfunction (MGD). MGD is the major cause of dry eye disease (DED), which affects 40 million people in the USA and has no cure. Azithromycin (AZM) is a PLD-inducing antibiotic that is used off-label to treat MGD, and is presumably effective because it suppresses the MGD-associated conjunctival inflammation (i.e. posterior blepharitis) and growth of lid bacteria. We hypothesize that AZM can act directly to promote the function of human meibomian gland epithelial cells by inducing PLD in these cells, characterized by the accumulation of lipids and lysosomes. Immortalized human meibomian gland epithelial cells (HMGEC) were cultured with or without azithromycin for 5 days. Cells were evaluated for cholesterol (Filipin) and neutral lipid (LipidTox) staining, as well as the appearance of lysosomes (LysoTracker) and lamellar bodies (transmission electron microscopy, TEM). The lipid composition of cellular lysates was analyzed by high performance thin-layer chromatography. Our findings demonstrate that AZM stimulates the accumulation of free cholesterol, neutral lipids and lysosomes in HMGEC. This AZM-induced increase of neutral lipid content occurred predominantly within lysosomes. Many of these vesicles appeared to be lamellar bodies by TEM, which is the characteristic of PLD. Our findings also show that AZM promotes an accumulation of free and esterified cholesterol, as well as phospholipids in HMGECimmortalized. Our results support our hypothesis and confirm the beneficial effect of PLD induced by AZM on HMGEC. Our discovery reveals a new potential use of PLD-inducing drugs, and makes this adverse effect a beneficial effect.

1. Introduction

Drug-induced phospholipidosis (PLD) is an excessive intracellular accumulation of phospholipid, characterized by the formation of distinct, onion-shaped secretory lysosomes, termed lamellar bodies (Anderson and Borlak, 2006). Drug-induced PLD can be caused by many drugs, especially cationic amphiphilic drugs (CADs) (Anderson and Borlak, 2006). It is considered to be a major problem for the pharmaceutical industry because of potential toxicity and the huge expense to screen out PLD-inducing drugs each year (Shayman and Abe, 2013). The development of some lead compounds has been terminated when PLD was seen in certain organs in clinical trials (Shayman and Abe, 2013). The mechanism of PLD has been linked to enhanced cholesterol synthesis (Lowe et al., 2012), but its significance to humans is unclear. Although it is generally considered as a "poisonous" effect that the pharmaceutical industry wants to eliminate, we think this lipid accumulation effect may be beneficial in the treatment of meibomian gland dysfunction (MGD).

MGD is the most common cause of dry eye disease (DED), which afflicts tens of millions of people in the United States, and is one of the leading reasons for patient visits to eye care practitioners (The International Dry Eye Workshop, 2007), Meibomian glands normally produce abundant lipids (e.g. cholesterol and phospholipids), that accumulate in lysosomes, are secreted in a holocrine manner into lateral ducts, and ultimately released onto the ocular surface (Green-Church et al., 2011; Knop et al., 2011) (Unpublished results). These secretions enhance the stability and prevent the evaporation of the tear film, thereby playing a critical role in the wellbeing of the eye (Green-Church et al., 2011; Knop et al., 2011). However, MGD, and the associated lipid deficiency, disrupts this

Abbreviations: PLD, phospholipidosis; CADs, cationic amphiphilic drugs; MGD, meibomian gland dysfunction; DED, dry eye disease; AZM, azithromycin; HMGEC, Immortalized human meibomian gland epithelial cells; TEM, transmission electron microscopy; CE, cholesterol ester; FC, free cholesterol; PE, phosphatidylethanolamine; PC, phosphatidylcholine; PI, phosphatidylinositol.

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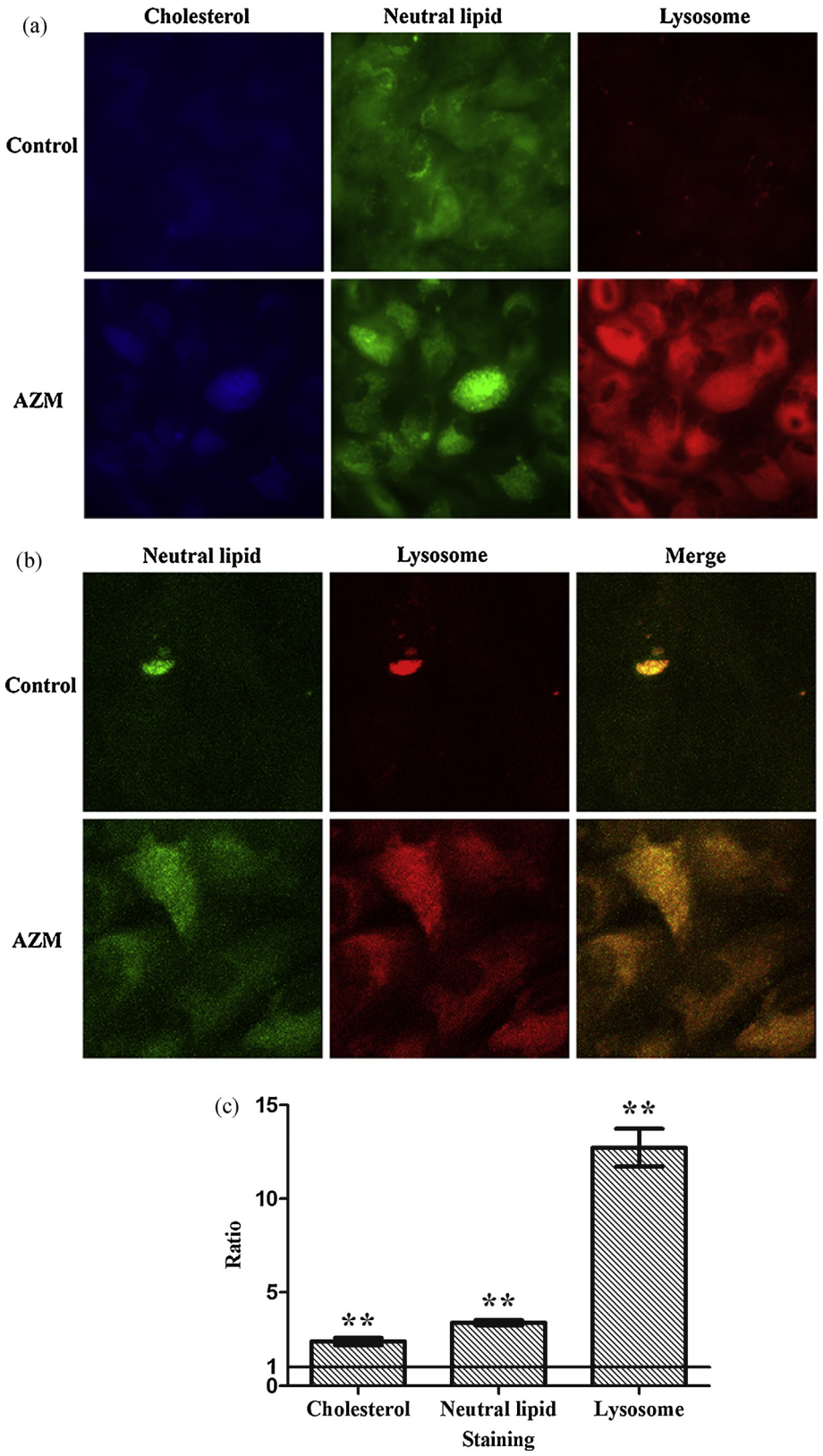

Fig. 1. Effect of AZM on intracellular accumulation of lipids and lysosomes. Cells were treated with vehicle or 10 μg/ml AZM for 5 days. (a) The blue color is Filipin stain indicating free cholesterol, green color is LipidTOX green neutral lipid stain, and red color is LysoTracker red stain indicating lysosomes. Images were obtained with a fluorescent microscope. (b) Cells were stained as in (a), and images were obtained with a confocal microscope in order to show colocalization of neutral lipids and lysosomes. (c) The fluorescence intensity was measured by using ImageJ; control image intensity was set to 1 and data (mean ± SE) are reported as fold-change compared to control values. $^{**}p < 0.005$ versus control. Free choleserol staining was repeated 3 times, neutral lipid staining 5 times and lysosome staining 4 times. All results shown are from a single experiment.