Published in final edited form as:

Curr Eye Res. 2018 June ; 43(6): 683–688. doi:10.1080/02713683.2017.1418894.

The Effect of Solithromycin, a Cationic Amphiphilic Drug, on the Proliferation and Differentiation of Human Meibomian Gland Epithelial Cells

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Abstract

Purpose: We previously discovered that azithromycin (AZM) acts directly on immortalized human meibomian gland epithelial cells (IHMGEs) to stimulate their lipid and lysosome accumulation and overall differentiation. We hypothesize that this phospholipidosis-like effect is due to AZM's cationic amphiphilic drug (CAD) nature. If our hypothesis is correct, then other CADs (e.g., solithromycin [SOL]) should be able to duplicate AZM's action on IHMGEs. Our purpose was to test this hypothesis.

Materials and Methods: IHMGEs were cultured in the presence of vehicle or SOL (2, 10, or 20 µg/ml) for up to 7 days under proliferating or differentiating conditions. Positive (epidermal growth factor and bovine pituitary extract for proliferation; AZM for differentiation) and negative (vehicle) controls were included with the experiments. IHMGEs were evaluated for cell number, neutral lipid content, and lysosome accumulation.

Results: Our results demonstrate that SOL induces a rapid and dose-dependent increase in the accumulation of neutral lipids and lysosomes in IHMGEs. The lysosomal effects were most prominent with the 10 and 20 µg/ml doses, and occurred earlier (i.e., 1 day) with SOL than with the AZM (10 µg/ml) control. The effects of SOL and AZM on IHMGE differentiation were essentially the same after 3 days of culture. SOL did not influence the proliferation of IHMGEs during a 7-day time period.

Conclusions: Our results support our hypothesis that SOL, a CAD, is able to reproduce AZM's impact on lysosome and lipid accumulation, as well as the differentiation, of IHMGEs. The effect of SOL on lysosome appearance was faster than that of AZM.

Keywords

Solithromycin; azithromycin; cationic amphiphilic drug; phospholipidosis; meibomian gland dysfunction; dry eye disease

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Declaration of interests

This study was sponsored by Cempra Pharmaceuticals.

Introduction

Meibomian gland dysfunction (MGD) is the leading cause of dry eye disease (DED)^{1–6}, which afflicts hundreds of millions of people throughout the world.^{7–9} Normally, meibomian glands (MGs) generate abundant lipids that accumulate in lysosomes, are secreted in a holocrine manner into lateral ducts, and are ultimately released onto the ocular surface.^{4,10} This glandular secretion, termed meibum, provides a clear optical surface for the cornea, interferes with bacterial colonization, and retards tear overflow.^{1,7–11} Meibum also promotes the stability and prevents the evaporation of the tear film, thereby playing a critical role in the health of the ocular surface.^{1,10,12–15}

However, MGD, and the resulting meibum insufficiency, destabilizes the tear film, increases its osmolarity and evaporation, and leads to DED.^{1–6,12,14,16–19} The most frequent cause of human MGD is excretory duct obstruction, due to reduced meibum quality and hyperkeratinization of the terminal duct epithelium.^{1,20–27} This obstruction may lead to cystic dilatation of glandular ducts, and the atrophy and loss of human MG epithelial cells (HMGECs).^{1,22,28} MGD may also facilitate bacterial growth on the lid margin^{29,30} and promote inflammation in the adjacent conjunctiva (i.e., posterior blepharitis).^{1,31,32}

The most frequent pharmaceutical treatment in the United States for the management of MGD is the off-label use of azithromycin (AZM).³³ This macrolide antibiotic was thought to be effective due to its anti-bacterial and anti-inflammatory actions, which may suppress the MGD-associated growth of lid bacteria and posterior blepharitis.^{34–37} However, we discovered that AZM also has the ability to act directly on HMGECs to stimulate their differentiation, enhance the quality and quantity of their lipid production, and promote their holocrine secretion.³⁸ We hypothesize that this AZM capability is due to its cationic amphiphilic drug (CAD) nature and reflects a phospholipidosis (PLD)-like effect.³⁹ CADs feature a hydrophobic ring structure linked to a hydrophilic domain with a cationic amine group.⁴⁰ Drug-induced PLD, in turn, is characterized by an intracellular accumulation of cholesterol and phospholipids, and the formation of distinct, onion-shaped secretory lysosomes, termed lamellar bodies.⁴⁰

In support of our hypothesis, we discovered that AZM stimulates the accumulation of free and esterified cholesterol, neutral lipids, phospholipids, and lysosomes in immortalized (I) HMGECs. This increase of neutral lipid content occurs predominantly within lysosomes, many of which appear to be onion-shaped lamellar bodies.³⁹ This intracellular accumulation of phospholipids and the concurrent development of lamellar bodies are the hallmark features, and indeed the gold standard, for identifying PLD.^{41,42}

Given our research findings, we further hypothesize that other CADs should be able to reproduce AZM's action on IHMGECs and potentially serve as a therapy for human MGD. To begin to test this hypothesis, we sought to determine whether solithromycin (SOL), a CAD, mirrors AZM activity, and stimulates the differentiation of IHMGECs. In addition, because it is possible that PLD-inducing drugs may elicit toxic effects in other tissues⁴³, we assessed the impact of SOL on the survival and proliferation of IHMGECs.

Materials and methods

IHMGEs were cultured under differentiating and proliferating conditions, according to published protocols.^{44–46} For differentiation studies IHMGEs were grown on glass coverslips in 6-well plates until ~80% confluent, then cultured in Dulbecco's modified Eagle's medium (DMEM)/Ham's F12 (50:50; Thermo Fisher Scientific, Waltham, MA), supplemented with 10% fetal bovine serum (Thermo Fisher Scientific), SOL (2, 10, or 20 µg/mL; Cemptra), or AZM (10 µg/mL; Santa Cruz Biotechnology, Dallas, TX) for up to 7 days. Cells were also treated with equivalent concentrations of both ethanol (AZM vehicle) and dimethyl sulfoxide (DMSO; SOL vehicle). At experimental termination, total cellular neutral lipid and lysosome accumulations were evaluated by staining cells with LipidTOX green neutral lipid stain (Thermo Fisher Scientific) and LysoTracker® Red DND-99 (Thermo Fisher Scientific), a fluorescent probe designed for labeling acidic organelles (e.g., lysosomes), as previously reported.^{38,39,47,48} Coverslips were mounted on slides with ProLong Gold antifade reagent with 4',6-diamidino-2-phenylindole (DAPI; Thermo Fisher Scientific) for DNA staining and allowed to dry overnight. Images were captured with a Nikon fluorescent TS100 phase contrast microscope and staining intensities were quantified with ImageJ.⁴⁹ Data were analyzed by ANOVA and Newman–Keuls multiple comparisons tests by using Prism 5 software (GraphPad Software, Inc., La Jolla, CA). Image quantification was performed using ImageJ (<http://rsbweb.nih.gov/ij/index.html>). Eight images were analyzed for each condition and background intensity was subtracted.

For proliferation experiments, IHMGEs were seeded in 24-well plates in keratinocyte serum-free medium (KSFM) supplemented with epidermal growth factor (EGF; 5 µg/mL) and bovine pituitary extract (BPE; 50 µg/mL). Cells were then washed and the media were changed to unsupplemented (basal) KSFM with or without SOL (2, 10, or 20 µg/mL), AZM (10 µg/mL), or fresh KSFM containing EGF and BPE. After 7 days, cell numbers were enumerated using a hemocytometer. The EGF- and BPE-treated cells were positive controls for proliferation in these studies.⁴⁵

Results

Dose-dependent effect of SOL on lipid and lysosome accumulation in IHMGEs

To determine whether SOL stimulates the accumulation of neutral lipids and lysosomes in IHMGEs, we cultured cells in the presence of vehicles (i.e., ethanol and DMSO), SOL (2, 10, or 20 µg/mL), or the positive-control AZM (10 µg/mL) for up to 7 days under differentiating conditions. Our results demonstrate that SOL, like AZM, elicits a marked increase in the neutral lipid content and lysosome appearance in IHMGEs (Figure 1). As with AZM, this SOL-induced rise in lipid levels occurred predominantly within lysosomes (Figure 1).

The influence of SOL on IHMGE differentiation was dose-dependent. As shown in Figure 2, we observed a dramatic increase in both neutral lipids and lysosomes in the IHMGEs treated with 10 µg/mL and 20 µg/mL SOL concentrations. In contrast, the 2 µg/mL SOL dosage may have induced a slight rise in neutral lipid accumulation within lysosomes, but

this was difficult to determine, as the signal intensity was similar to that in vehicle-treated controls.

Time-course of SOL's influence on lipid and lysosome accumulation in IHMGECs

To examine the time-dependence of the SOL stimulatory effect on IHMGEC differentiation, as well as to assess whether SOL activity occurs in a time-frame analogous to that of AZM, we cultured cells in the presence of vehicles (i.e., ethanol and DMSO), SOL (10 µg/ml) or the positive-control AZM (10 µg/ml) for 1, 3, and 5 days under differentiating conditions.

As demonstrated in Figure 3, SOL induced a rapid and robust increase in lysosome accumulation. This effect, which occurred within 1 day, was unique to SOL and not duplicated by AZM. After 3 (Figure 3) and 5 days of treatment, both SOL and AZM had essentially the same upregulatory effects on the numbers of lysosomes in IHMGECs. With regard to neutral lipid accumulation in IHMGECs, the stimulatory actions of SOL and AZM followed a similar time course (Figure 3).

Impact of SOL on the proliferation of IHMGECs

To investigate whether SOL modulates the survival or proliferation of IHMGECs, we cultured cells in the presence of vehicles, SOL (2, 10 or 20 µg/ml), AZM (10 µg/ml), or the positive-control EGF and BPE for 7 days in KSFM media. Our findings demonstrate that cellular exposure to EGF and BPE caused, as anticipated, a significant increase in IHMGEC proliferation in serum-free media (Figure 4). However, neither SOL nor AZM altered the number of cells, as compared to that of the vehicle-treated control (Figure 4).

Discussion

Our results demonstrate that SOL induces a rapid and dose-dependent increase in the accumulation of neutral lipids and lysosomes in IHMGECs. The lysosomal effects were most prominent with the 10 and 20 µg/ml doses and occurred earlier (i.e., 1 day) with SOL than with the AZM positive control. The effects of SOL and AZM on IHMGEC differentiation were essentially the same after 3 days of culture. SOL did not influence the proliferation of IHMGECs in serum-free media during a 7-day time period. Our results support our hypothesis that SOL, a CAD, is able to reproduce AZM's impact on lysosome and lipid accumulation of IHMGECs. Of particular interest, the ability of SOL to act like a CAD on IHMGECs to stimulate their PLD-like maturation³⁹ cannot be duplicated by non-CAD compounds, such as the antibiotics tetracycline, doxycycline and minocycline⁴⁸, or omega 3 and 6 fatty acids.⁵⁰

The mechanism by which SOL promotes the differentiation of IHMGECs most likely involves a PLD-like effect. SOL is a novel fluoroketolide⁵¹ that is known to accumulate in other eukaryotic cells (e.g., macrophages, kidney epithelial cells)^{52,53}, become sequestered in lysosomes, and induce PLD.⁵³ This PLD process has been associated with enhanced cholesterol synthesis⁴³ and is characterized by an intracellular accumulation of cholesterol and phospholipids, as well as the formation of distinct secretory lysosomes called lamellar bodies.⁴⁰ A lamellar body is a form of lysosome specialized for lipid storage and secretion.^{54–58} These concentric vesicle-like lamellar bodies have been identified in IHMGECs

following exposure to AZM³⁹, and they appear to accumulate lipids in the meibomian gland.^{59,60} SOL, in turn, increases the neutral lipid level and lysosome number in IHMGECs, and this lipid accumulation occurs predominantly within the lysosomes.

It is noteworthy that the pharmaceutical industry has considered PLD a toxic effect that warrants elimination.⁶¹ In fact, the development of various lead compounds has been terminated after PLD was discovered in various tissues in clinical trials.⁶¹ Such termination is understandable when one considers that the PLD induced by certain aminoglycoside and macrolide antibiotics is linked to cellular apoptosis and necrosis.^{62,63} However, studies to date indicate that SOL exposure, when used in microbiologically relevant concentrations, shows no evidence of cellular toxicity or impairment of anti-microbial defense.⁵³ Indeed, at the concentrations used in our study, SOL had no toxic impact on IHMGECs. Rather, SOL elicited a positive differentiative effect.

The SOL-induced generation of lysosomes and the accumulation of lipids within these vesicles are similar to events that typically occur during the differentiation of HMGECS.⁴⁶ This cellular process appears to start with small, undifferentiated cells located in the periphery of meibomian gland acini. These cells possess large quantities of free ribosomes and mitochondria, and a poorly developed smooth endoplasmic reticulum (SER) and Golgi apparatus. As cells mature and begin their migration toward the lateral ductules, the SER and Golgi become more apparent, lysosomes are generated, and lipids begin to accumulate. Ultimately, cells terminally differentiate, a process associated with a marked increase in volume, a profusion of lipid-filled vesicles, and nuclear pyknosis. Cells then undergo holocrine secretion, which involves cellular autophagy, apoptosis, disintegration and the release of lipid-laden contents into the lateral ductules, the central duct, and eventually the ocular surface.^{1,60,64–67}

The capacity of SOL to stimulate this differentiative process of HMGECS may, like AZM, be beneficial for the treatment of MGD. Further, because SOL harbors both anti-bacterial⁶⁸ and anti-inflammatory⁶⁹ activities, it is possible that this antibiotic may also decrease the MGD-linked growth of lid bacteria and development of posterior blepharitis.^{34–37} However, whether SOL can duplicate the clinically significant ophthalmic effects of AZM *in vivo* remains to be determined.

Funding

This research was sponsored by Cempira Pharmaceuticals and supported by the Margaret S. Sinon Scholar in Ocular Surface Research Fund and the Guoxing Yao Research Fund.

References

1. Knop E, Knop N, Millar T, Obata H, Sullivan DA. The international workshop on meibomian gland dysfunction: report of the subcommittee on anatomy, physiology, and pathophysiology of the meibomian gland. *Invest Ophthalmol Vis Sci.* 2011;52:1938–78. doi:10.1167/iovs.10-6997c. [PubMed: 21450915]
2. Shimazaki J, Sakata M, Tsubota K. Ocular surface changes and discomfort in patients with meibomian gland dysfunction. *Arch Ophthalmol.* 1995;113:1266–70. [PubMed: 7575257]

3. Nelson JD, Shimazaki J, Benitez-del-Castillo JM, Craig JP, McCulley JP, Den S, Foulks GN. The international workshop on meibomian gland dysfunction: report of the definition and classification subcommittee. *Invest Ophthalmol Vis Sci.* 2011;52:1930–37. [PubMed: 21450914]
4. Lemp MA, Crews LA, Bron AJ, Foulks GN, Sullivan BD. Distribution of aqueous-deficient and evaporative dry eye in a clinic-based patient cohort: a retrospective study. *Cornea.* 2012;31:472–78. [PubMed: 22378109]
5. Schaumberg DA, Nichols JJ, Papas EB, Tong L, Uchino M, Nichols KK. The international workshop on meibomian gland dysfunction: report of the subcommittee on the epidemiology of, and associated risk factors for, MGD. *Invest Ophthalmol Vis Sci.* 2011;52:1994–2005. [PubMed: 21450917]
6. Nichols KK, Foulks GN, Bron AJ, Glasgow BJ, Dogru M, Tsubota K, Lemp MA, Sullivan DA. The international workshop on meibomian gland dysfunction: executive summary. *Invest Ophthalmol Vis Sci.* 2011;52:1922–29. [PubMed: 21450913]
7. Sullivan DA, Tsubota K, Dartt DA, Stern ME, Sullivan RM, Bromberg BB, editors. *Lacrimal gland, tear film and dry eye syndromes 3 In: Basic science and clinical relevance.* New York: Kluwer Academic/Plenum Press; 2002.
8. 2007 Report of the international dry eye workshop (DEWS). *Ocul Surf.* 2007;5:65–204.
9. Sullivan DA, Hammitt KM, Schaumberg DA, Sullivan BD, Begley CG, Gjorstrup P, et al. Report of the TFOS/ARVO symposium on global treatments for dry eye disease: an unmet need. *Ocul Surf.* 2012;10:108–16. [PubMed: 22482471]
10. Green-Church KB, Butovich I, Willcox M, Borchman D, Paulsen F, Barabino S, et al. The international workshop on meibomian gland dysfunction: report of the subcommittee on tear film lipids and lipid-protein interactions in health and disease. *Invest Ophthalmol Vis Sci.* 2011;52:1979–93. [PubMed: 21450916]
11. Tsai P, Evans JE, Green KM, Sullivan RM, Schaumberg DA, Richards SM, et al. Proteomic analysis of human meibomian gland secretions. *Br J Ophthalmol.* 2006;90:372–77. [PubMed: 16488965]
12. Tiffany JM. Physiological functions of the meibomian glands. *Prog Retinal Eye Res.* 1995;14:47–74.
13. Craig JP, Tomlinson A. Importance of the lipid layer in human tear film stability and evaporation. *Optom Vis Sci.* 1997;74:8–13. [PubMed: 9148269]
14. Bron AJ, Tiffany JM, Gouveia SM, Yokoi N, Voon LW. Functional aspects of the tear film lipid layer. *Exp Eye Res.* 2004;78:347–60. [PubMed: 15106912]
15. McCulley JP, Shine WE. Meibomian gland function and the tear lipid layer. *Ocul Surf.* 2003;1:97–106. [PubMed: 17075642]
16. Foulks GN, Bron AJ. Meibomian gland dysfunction: a clinical scheme for description, diagnosis, classification, and grading. *Ocul Surf.* 2003;1:107–26. [PubMed: 17075643]
17. Bron AJ, Sci FM, Tiffany JM. The contribution of meibomian disease to dry eye. *Ocul Surf.* 2004;2:149–65. [PubMed: 17216085]
18. McCulley JP, Shine W. A compositional based model for the tear film lipid layer. *Trans Am Ophthalmol Soc.* 1997;95:79–88. [PubMed: 9440164]
19. Mathers WD, Lane JA. Meibomian gland lipids, evaporation, and tear film stability. *Adv Exp Med Biol.* 1998;438:349–60. [PubMed: 9634908]
20. Krenzer KL, Dana MR, Ullman MD, Cermak JM, Tolls BD, Evans JE, et al. Effect of androgen deficiency on the human meibomian gland and ocular surface. *J Clin Endocr Metab.* 2000;85:4874–82. [PubMed: 11134156]
21. Knop E, Knop N, Brewitt H, Pleyer U, Rieck P, Seitz B, et al. [Meibomian glands: part III. Dysfunction - argument for a discrete disease entity and as an important cause of dry eye]. *Ophthalmologe.* 2009;106:966–79. [PubMed: 19941140]
22. Knop E, Knop N. Meibomian glands: part IV. Functional interactions in the pathogenesis of meibomian gland dysfunction (MGD). *Ophthalmologe.* 2009;106:980–87. [PubMed: 19941141]
23. Gutgesell VJ, Stern GA, Hood CI. Histopathology of meibomian gland dysfunction. *Am J Ophthalmol.* 1982;94:383–87. [PubMed: 7124880]

24. Jester JV, Nicolaides N, Kiss-Palvolgyi I, Smith RE. Meibomian gland dysfunction. II. The role of keratinization in a rabbit model of MGD. *Invest Ophthalmol Vis Sci.* 1989;30:936–45. [PubMed: 2470694]
25. Anatomy Obata H. and histopathology of human meibomian gland. *Cornea.* 2002;21:S70–74. [PubMed: 12484702]
26. Sullivan BD, Evans JE, Dana MR, Sullivan DA. Influence of aging on the polar and neutral lipid profiles in human meibomian gland secretions. *Arch Ophthalmol.* 2006;124:1286–92. [PubMed: 16966624]
27. Miyake H, Oda T, Katsuta O, Seno M, Nakamura M. Meibomian gland dysfunction model in hairless mice fed a special diet with limited lipid content. *Invest Ophthalmol Vis Sci.* 2016;57:3268–75. [PubMed: 27327582]
28. Obata H, Horiuchi H, Miyata K, Tsuru T, Machinami R. [Histopathological study of the meibomian glands in 72 autopsy cases]. *Nippon Ganka Gakkai Zasshi.* 1994;98:765–71. [PubMed: 7942338]
29. Dougherty JM, McCulley JP. Comparative bacteriology of chronic blepharitis. *Br J Ophthalmol.* 1984;68:524–28. [PubMed: 6743618]
30. Dougherty JM, McCulley JP. Bacterial lipases and chronic blepharitis. *Invest Ophthalmol Vis Sci.* 1986;27:486–91. [PubMed: 3957566]
31. Messmer EM, Torres SE, Mackert MI, Zapp DM, Kampik A. [In vivo confocal microscopy in blepharitis]. *Klin Monatsbl Augenheilkd.* 2005;222:894–900. [PubMed: 16308822]
32. Chao W, Belmonte C, Benitez Del Castillo JM, Bron AJ, Dua HS, Nichols KK, et al. Report of the inaugural meeting of the TFOS i(2) = initiating innovation series: targeting the unmet need for dry eye treatment. *Ocul Surf.* 2016;14:264–316. [PubMed: 26774910]
33. Lemp MA, Nichols KK. Blepharitis in the United States 2009: a survey-based perspective on prevalence and treatment. *Ocul Surf.* 2009;7:S1–S14. [PubMed: 19383269]
34. Foulks GN, Borchman D, Yappert M, Kakar S. Topical azithromycin and oral doxycycline therapy of meibomian gland dysfunction: a comparative clinical and spectroscopic pilot study. *Cornea.* 2013;32:44–53. [PubMed: 22668581]
35. Foulks GN, Borchman D, Yappert M, Kim SH, McKay JW. Topical azithromycin therapy for meibomian gland dysfunction: clinical response and lipid alterations. *Cornea.* 2010;29:781–88. [PubMed: 20489573]
36. Geerling G, Tauber J, Baudouin C, Goto E, Matsumoto Y, O'Brien T. The international workshop on meibomian gland dysfunction: report of the subcommittee on management and treatment of meibomian gland dysfunction. *Invest Ophthalmol Vis Sci.* 2011;52:2050–64. [PubMed: 21450919]
37. Kashkouli MB, Fazel AJ, Kiavash V, Nojomi M, Ghiasian L. Oral azithromycin versus doxycycline in meibomian gland dysfunction: a randomised double masked open label clinical trial. *Br J Ophthalmol.* 2015;99:199–204. [PubMed: 25138765]
38. Liu Y, Kam WR, Ding J, Sullivan DA. Effect of azithromycin on lipid accumulation in immortalized human meibomian gland epithelial cells. *JAMA Ophthalmol.* 2014;132:226–28. [PubMed: 24357250]
39. Liu Y, Kam WR, Ding J, Sullivan DA. One man's poison is another man's meat. Using azithromycin-induced phospholipidosis to promote ocular surface health. *Toxicology.* 2014;320:1–5. [PubMed: 24613571]
40. Anderson N, Borlak J. Drug-induced phospholipidosis. *FEBS Lett.* 2006;580:5533–40. [PubMed: 16979167]
41. Sadrieh N 2010 The regulatory challenges of drug-induced phospholipidosis. FDA website <http://www.fda.gov/downloads/advisory-committees/committeesmeetingmaterials/drugs/advisorycommitteeofpharmaceuticalscienceandclinicalpharmacology/ucm210798.pdf>
42. Reasor MJ, Kacew S. Drug-induced phospholipidosis: are there functional consequences? *Exp Biol Med (Maywood).* 2001;226:825–30. [PubMed: 11568304]
43. Lowe R, Mussa HY, Nigsch F, Glen RC, Mitchell JB. Predicting the mechanism of phospholipidosis. *J Cheminform.* 2012;4:2. [PubMed: 22281160]

44. Liu S, Hatton MP, Khandelwal P, Sullivan DA. Culture, immortalization, and characterization of human meibomian gland epithelial cells. *Invest Ophthalmol Vis Sci.* 2010;51:3993–4005. [PubMed: 20335607]
45. Liu S, Kam WR, Ding J, Hatton MP, Sullivan DA. Effect of growth factors on the proliferation and gene expression of human meibomian gland epithelial cells. *Invest Ophthalmol Vis Sci.* 2013;54(4):2541–50. [PubMed: 23493293]
46. Sullivan DA, Liu Y, Kam WR, Ding J, Green KM, Shaffer SA, et al. Serum-induced differentiation of human meibomian gland epithelial cells. *Invest Ophthalmol Vis Sci.* 2014;55:3866–77. [PubMed: 24867579]
47. Liu Y, Ding J. The combined effect of azithromycin and insulin-like growth factor-1 on cultured human meibomian gland epithelial cells. *Invest Ophthalmol Vis Sci.* 2014;55:5596–601. [PubMed: 25125598]
48. Liu Y, Kam WR, Ding J, Sullivan DA. Can tetracycline antibiotics duplicate the ability of azithromycin to stimulate human meibomian gland epithelial cell differentiation? *Cornea.* 2015;34:342–46. [PubMed: 25611398]
49. <http://rsbweb.nih.gov/ij/index.html>
50. Liu Y, Kam WR, Sullivan DA. Influence of omega 3 and 6 fatty acids on human meibomian gland epithelial cells. *Cornea.* 2016;35:1122–26. [PubMed: 27227397]
51. Buege MJ, Brown JE, Aitken SL. Solithromycin: a novel ketolide antibiotic. *Am J Health Syst Pharm.* 2017;74:875–87. [PubMed: 28432048]
52. Lemaire S, Van Bambeke F, Tulkens PM. Cellular accumulation and pharmacodynamic evaluation of the intracellular activity of CEM-101, a novel fluoroketolide, against staphylococcus aureus, listeria monocytogenes, and Legionella pneumophila in human THP-1 macrophages. *Antimicrob Agents Chemother.* 2009;53:3734–43. [PubMed: 19564365]
53. <http://www.cempra.com/common/pdf/Posters/ICAAC%19202010%20A1-686%20Das%20Tulkens%20CEM101%20Final%208-26-10.pdf>
54. Rabionet M, Gorgas K, Sandhoff R. Ceramide synthesis in the epidermis. *Biochimica Et Biophysica Acta.* 2014;1841:422–34. [PubMed: 23988654]
55. Schmitz G, Muller G. Structure and function of lamellar bodies, lipid-protein complexes involved in storage and secretion of cellular lipids. *J Lipid Res.* 1991;32:1539–70. [PubMed: 1797938]
56. Weaver TE, Na CL, Stahlman M. Biogenesis of lamellar bodies, lysosome-related organelles involved in storage and secretion of pulmonary surfactant. *Semin Cell Dev Biol.* 2002;13:263–70. [PubMed: 12243725]
57. Baronas ET, Lee JW, Alden C, Hsieh FY. Biomarkers to monitor drug-induced phospholipidosis. *Toxicol Appl Pharmacol.* 2007;218:72–78. [PubMed: 17156806]
58. Dietl P, Haller T, Mair N, Frick M. Mechanisms of surfactant exocytosis in alveolar type II cells in vitro and in vivo. *News Physiol Sci.* 2001;16:239–43. [PubMed: 11572929]
59. Jester JV, Nicolaides N, Smith RE. Meibomian gland studies: histologic and ultrastructural investigations. *Invest Ophthalmol Vis Sci.* 1981;20:537–47. [PubMed: 7194327]
60. Sirigu P, Shen RL, Pinto Da Silva P. Human meibomian glands: the ultrastructure of acinar cells as viewed by thin section and freeze-fracture transmission electron microscopies. *Invest Ophthalmol Vis Sci.* 1992;33:2284–92. [PubMed: 1607240]
61. Shayman JA, Abe A. Drug induced phospholipidosis: an acquired lysosomal storage disorder. *Biochim Biophys Acta.* 2013;1831:602–11. [PubMed: 22960355]
62. El Mouedden M, Laurent G, Mingot-Leclercq MP, Tulkens PM. Gentamicin-induced apoptosis in renal cell lines and embryonic rat fibroblasts. *Toxicol Sci.* 2000;56:229–39. [PubMed: 10869472]
63. Servais H, Van Der Smitten P, Thirion G, Van Der Essen G, Van Bambeke F, Tulkens PM, et al. Gentamicin-induced apoptosis in LLC-PK1 cells: involvement of lysosomes and mitochondria. *Toxicol Appl Pharmacol.* 2005;206:321–33. [PubMed: 16039943]
64. Thody AJ, Shuster S. Control and function of sebaceous glands. *Physiol Rev.* 1989;69:383–416. [PubMed: 2648418]
65. Mesquita-Guimarães J, Coimbra A. Holocrine cell lysis in the rat preputial sebaceous gland. Evidence of autophagocytosis during cell involution. *Anat Rec.* 1976;186:49–67.

66. Mesquita-Guimarães J, Pignatelli D, Coimbra A. Autophagy during holocrine cell lysis in skin sebaceous glands. *J Submicrosc Cytol.* 1979;11:435–47.
67. Brandes D, Bertini F, Smith EW. Role of lysosomes in cellular lytic processes. II. Cell death during holocrine secretion in sebaceous cells. *Exp Mol Pathol.* 1965;4:245–65. [PubMed: 14300111]
68. Farrell DJ, Flamm RK, Sader HS, Jones RN. Results from the solithromycin international surveillance program (2014). *Antimicrob Agents Chemother.* 2016;60:3662–68. [PubMed: 27044551]
69. http://www.cempra.com/common/pdf/posters/IDWeek%19202012_1613_Ito_Soli%20and%20COPD_FINAL.pdf

Figure 1.

Effect of SOL on neutral lipid and lysosome accumulation in IHMGECs. Cells ($n = 3$ wells/treatment; $n = 2$ experiments) were treated with vehicles (i.e. ethanol and DMSO), SOL (10 μ g/ml) or AZM (10 μ g/ml) for 5 days under differentiating conditions. Neutral lipids were stained with LipidTOX (green), lysosomes with LysoTracker (red), and nuclei with DAPI (blue). A. The appearance of lysosome and neutral lipid staining after 5 days of antibiotic treatment. All images are 400X magnification. B. Fluorescence intensity of the neutral lipid staining was quantified using Image J. C. Fluorescence intensity of the lysosome staining.

Data shown are mean fluorescence intensity $\pm$ SEM. ** indicates $p < 0.01$ compared to control.

Figure 2.

Impact of different doses of SOL on neutral lipid and lysosome accumulation in IHMGECs.

Cells ($n = 3$ wells/treatment; $n = 4$ experiments) were cultured in the presence of vehicles (i.e. ethanol and DMSO), SOL (2, 10, or 20 µg/ml) or AZM (10 µg/ml) for 7 days under differentiating conditions. The targets of the LipidTOX, LysoTracker and DAPI stains are explained in the Methods and the legend for Figure 1. All images are 400X magnification.

B. Fluorescence intensity of the neutral lipid staining was quantified using ImageJ. C.

Fluorescence intensity of the lysosome staining. Data shown are mean fluorescence intensity $\pm$ SEM. ** indicates $p < 0.01$ compared to control, † indicates $p < 0.05$ compared to azithromycin-treated cells.

Figure 3.

Time-dependent effect of SOL on neutral lipid and lysosome accumulation in IHMGECs. Cells ($n = 3$ wells/treatment; $n = 2$ experiments) were exposed to vehicles (i.e. ethanol and DMSO), SOL ($10 \mu\text{g/ml}$) or AZM ($10 \mu\text{g/ml}$) for 1 and 3 days under differentiating conditions. A. The appearance of lysosome and neutral lipid staining after antibiotic treatments. B. Fluorescence intensity of neutral lipid staining on day 1 was measured using ImageJ. C. Fluorescence intensity of the lysosome staining on day 1. D. Fluorescence intensity of neutral lipid staining on day 3. E. Fluorescence intensity of the lysosome staining on day 3. Data shown are mean fluorescence intensity $\pm$ SEM. * indicates $p < 0.05$

compared to control, ** indicates $p < 0.01$ compared to control, † indicates $p < 0.05$ compared to azithromycin-treated cells.

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Figure 4.

Influence of SOL on IHMGEC proliferation. Cells ($n = 3$ wells/treatment; $n = 4$ experiments) were cultured with vehicles, SOL (2, 10 or 20 µg/ml), AZM (10 µg/ml), or the positive-control EGF and BPE for 7 days in KSFM media. Data from one experiment are shown. Columns depict the mean $\pm$ standard error. * indicates $p < 0.05$.